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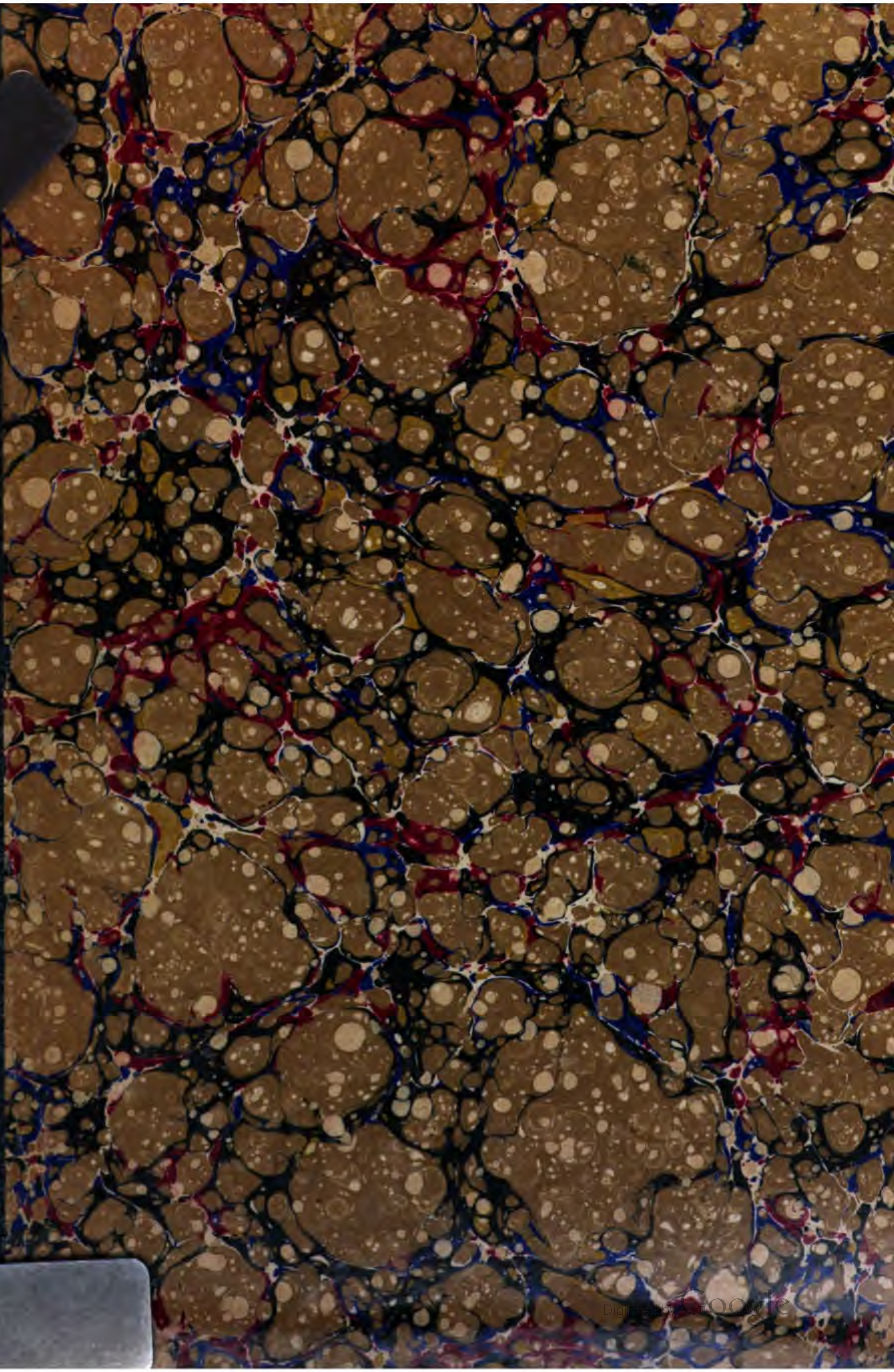

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CONTROL TESTS OF A RESPIRATION CALORIMETER.

By FRANCIS G. BENEDICT, J. A. RICHE, and L. E. EMMES.

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THE two respiration calorimeters described by Benedict and Carpenter[1] have been subjected to numerous tests as to the capability of the apparatus for measuring accurately heat, water vapor, carbon dioxide, and oxygen, by developing measured amounts of heat electrically, or burning known amounts of alcohol inside the chamber. The results obtained in control experiments of six or eight hours have been very satisfactory, but in experiments with man the measurements are often made in periods as short as one hour. Since any irregularities in measurement, either of the heat developed inside the chamber or of the heat measured by the chamber, may easily be averaged throughout a period of six or eight hours and still not affect materially the result for the whole period, it is necessary to prove that the apparatus measures accurately all four factors during a period as short as one hour before the results of one-hour experiments with man can be considered as reliable and accurate. Two kinds of tests have therefore been made, — the first in order to determine the accuracy of the apparatus as a calorimeter, and the second to test its efficiency both as a respiration apparatus and as a calorimeter.

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[1] BENEDICT and CARPENTER: Respiration calorimeters for studying the respiratory exchange and energy transformations of man. Carnegie Institution of Washington Publication No. 123, 1910.

ELECTRICAL CONTROL TESTS OF THE MEASUREMENT OF HEAT ELIMINATION.

In order to test the apparatus as a calorimeter, a constant current from the adjacent power house of the Harvard Medical School was used to develop heat inside the chamber. This current was first passed through a carefully calibrated mil-ammeter, then through an 81.3 ohm resistance coil of manganin wire, which was wound on a wooden frame and suspended in the calorimeter chamber in such a way as not to come in contact with the metal wall. A voltmeter outside of the chamber, also carefully calibrated, was connected with the terminals of the resistance coil, and the drop in potential could thus be accurately measured. The voltmeter was ordinarily not connected except at the time of reading, when the switch was closed for an instant; the mil-ammeter was shunted so that, unless desired, very little of the current need pass through the instrument itself, except when the switch was open.

The current from the power house is remarkably steady, the fluctuations not exceeding 1 or 2 volts in the 110-volt circuit during the course of twenty-four hours. The current was, however, passed through a small variable resistance in the main circuit before entering the chamber, so that, by hand regulation, even the slight fluctuations in voltage naturally occurring in the power house were eliminated by varying the resistance.

The voltmeter used was an instrument reading from 0 to 150 volts, while the mil-ammeter indicates from 0 to 1500 milli-amperes. Both instruments were especially adjusted and calibrated in the laboratory of the Weston Electrical Instrument Company, and subsequently tested and calibrated by the National Bureau of Standards. We are much indebted to Mr. S. C. Dinsmore of the Weston Electrical Instrument Company, formerly connected with the researches with the respiration calorimeter at Wesleyan University, for his kindness in looking after the careful calibration of these instruments.

The readings on these two instruments were used in calculating the theoretical amounts of heat developed inside the calorimeter chamber by means of the formula $I \times E \times t \times 0.2385$, in which I is the current in amperes, E, the electromotive force, t, the time in seconds, and 0.2385, the factor used to convert the watt-seconds to calories.

The amount of heat measured by the calorimeter was calculated from the records of (1) the mass of water passing through the heat-absorbing system; (2) the average differences between the temperatures of the cooling current of water as it entered and left the calorimeter as measured on two carefully calibrated mercurial thermometers read to 0.01°; and (3) the variations in temperature of the calorimeter as recorded on two series of electrical resistance thermometers, which indicated both the temperature of the copper wall and also the temperature of the air. The results of a sample test of this type are shown herewith in tabular form.

TABLE I.

RECORD OF HEAT DEVELOPED IN ELECTRICAL CHECK EXPERIMENT WITH CHAIR CALORIMETER. OCT. 14, 1909 (1-HOUR PERIODS).

Period.	Theory.	Found.	Percentage found.
	cal.	cal.	per cent.
First	87.1	86.9	99.8
Second	87.4	88.0	100.7
Third	86.9	86.6	99.7
Fourth	86.9	86.3	99.3
Total	348.3	347.8	99.9

DETERMINATION OF THE HYDROTHERMAL EQUIVALENT OF A RESPIRATION CALORIMETER.

The determination of the hydrothermal equivalent of a calorimeter large enough for experiments with man presents certain peculiar features that are worthy of special consideration. The type of calorimeter here described is unlike all others in that it is built on the adiabatic principle, and consequently a rise in temperature during an experiment results in part from the heat given up to the apparatus by the source of heat inside the chamber and in part from the heat supplied to the apparatus by an arbitrarily controlled source of heat outside the chamber. The exact apportionment of this heat is a problem of unusual interest.

The apparatus is constructed with an inner wall of copper, about which is a wall of zinc, with a 75 mm. air space between the two walls. Outside the zinc wall is a second air space, which is in turn surrounded by hair felt and asbestos lumber. The copper and zinc walls are attached to upright bars of structural steel channel iron, but the copper wall does not come in contact with the steel, owing to insulation by means of strips of asbestos wood. Between the two walls is a system of thermal junctions by which any differences in temperature are readily detected, while between the hair felt and the zinc wall is a system of heating wires and cooling pipes by which the temperature inside the chamber may be arbitrarily controlled.

To provide for the removal of the heat produced artificially or by the subject, a heat-absorbing apparatus is installed within the chamber. This consists of a continuous brass pipe, to which are soldered at intervals copper disks which greatly increase the heat-absorbing surface. By varying the temperature of the water which flows through this pipe, an attempt is made so to regulate the amount of heat brought away that it will exactly equal the heat produced and thus insure a constant temperature inside the chamber. It is, however, practically impossible in an experiment, especially with man, to secure such constant conditions at the beginning and end of each period, and there are slight differences in temperature which accompany certain kinds of experimenting. While ordinarily these differences amount to only a few hundredths of a degree, yet they may amount to 0.1° or 0.2°, and accordingly some information with regard to the hydrothermal equivalent of the apparatus is highly desirable. The methods for determining this hydrothermal equivalent are at first sight somewhat complex, as a rise in temperature of the calorimeter may result from several distinct causes. Since it has been proved, however, that the apparatus measures most accurately the amount of heat generated in the apparatus electrically, the hydrothermal equivalent may be determined by means of an electrical check experiment.

At certain times during such an experiment the temperature of the ingoing water is increased so that the heat, instead of being brought away by the water current, is absorbed by the walls of the chamber and thus the temperature of the apparatus slowly raised. Simultaneously a careful control is maintained of the thermo-electric system between the two metal walls and sufficient heat is applied to the outer wall to prevent heat passing through from the copper to the zinc wall, thus avoiding a cooling correction. When the temperature of the apparatus has risen to approximately the point required, the temperature of the ingoing water is lowered so as to bring away the heat as rapidly as it is generated and the chamber is held at a constant temperature for the next period. A control experiment made at this last temperature level should show an agreement between the theoretical heat developed electrically and the heat measured, while in the previous period there should be a distinct deficiency in the heat measured as compared with the heat produced. This deficiency will obviously be a measure of the heat absorbed by the calorimeter.

Two final tests are made, in the first of which the temperature of the water current is so regulated as to bring away somewhat more heat than is being generated and the calorimeter cools slowly, precaution being taken to likewise cool the zinc wall so that there will be no temperature difference between the two walls. At the end of the period, when the temperature has fallen to the desired point and a constant temperature is secured, a final control test is made, during which time the heat generated should exactly equal the heat measured. During the preceding period, however, while the calorimeter is cooling, there will obviously be more heat measured than was generated, the excess heat being given up by the calorimeter.

The temperature of the chamber is very accurately recorded by means of a series of resistance thermometers, one set being suspended in the air about 3.5 centimetres from the copper wall, and a second set being enclosed in small copper boxes securely attached to the wall, thereby indicating the temperature of the copper wall rather than of the air in the vicinity of the boxes.

The difference between the amounts of heat measured and of the heat introduced when the temperature is falling represents the heat lost by the calorimeter during the period, and if this is divided by the temperature fall, it will give in calories the heat yielded by the calorimeter per degree of change in temperature; from these data one can easily obtain the hydrothermal equivalent. On the other hand, the deficiency in heat between that introduced and that actually measured during the rising period gives a measure of the heat absorbed, and this, divided by the temperature rise, also gives direct data for computing the hydrothermal equivalent. Theoretically, under like conditions, the measurement of heat absorbed in the rising period and of the heat given off in the falling period should agree. The results obtained in an experiment of this nature are given in the table herewith.

TABLE II.

RECORD OF HEAT IN ELECTRICAL CHECK EXPERIMENT MADE TO DETERMINE THE HYDROTHERMAL EQUIVALENT OF THE CHAIR CALORIMETER, NOV. 10, 1909.

Period.	Temp. of calorimeter.	Theory.	Found.	Variation from theory.	Heat per degree of change in temperature of calorimeter.
	° C.	cal.	cal.	cal.	cal.
10.28 A. M.	19.95	....	....	.....	...
10.28 A. M.-11.28 "	19.95	84.7	83.2	− 1.5	...
11.28 " -12.28 P. M.	19.97	84.7	83.7	− 1.0	...
12.28 P. M.- 1.28 "	19.99	84.7	85.5	+ 0.8	...
1.28 " - 2.28 "	19.97	84.7	85.2	+ 0.5	...
2.28 " - 4.59 "	21.47	213.2	183.7	−29.5	19.7
4.59 " - 5.59 "	21.47	84.7	84.8	+ 0.1	...
5.59 " - 8.20 "	20.28	199.1	222.1	+23.0	19.4
8.20 " - 9.20 "	20.32	84.7	86.6	+ 1.9	...
Average	....	...	...	...	19.5

From the data given in this table, it is seen that the heat absorbed or given off by the calorimeter during the rising and falling periods was essentially the same. Since the amount of heat required to raise the temperature of the calorimeter 1° under these conditions is 19.5 calories, *i. e.*, the amount of heat which would be required to raise 19.5 kg. of water 1°, the value for the hydrothermal equivalent of this calorimeter may accordingly be taken as 19.5 kg.

An attempt has also been made to compute roughly the hydrothermal equivalent of the calorimeter from the recorded weights of the copper wall and fixtures of the apparatus, but the numerous factors entering into the equalizing of the heat from the inner and outer sources make it practically impossible to compute this with any degree of accuracy. Furthermore, the value here obtained by direct experimentation has a much greater significance, as the conditions of experimenting during an electrical check test approximate very closely the conditions of experimenting with man. The value of 19.5 kg. found with this calorimeter represents, therefore, with a degree of accuracy far within the limit of experimental error, the hydrothermal equivalent of the respiration calorimeter as used in experiments with man. A similar series of experiments on another somewhat larger calorimeter in the same laboratory showed a hydrothermal equivalent of approximately 21 kg.

The results of these electrical check experiments show that the calorimeter, when carefully manipulated, enables us to measure heat with an accuracy inside of 1 per cent for periods as short as one hour.

SIMULTANEOUS TESTS OF ACCURACY AS A RESPIRATION APPARATUS AND AS A CALORIMETER.

Since the ultimate object of using an apparatus of this type is to determine simultaneously four important factors of metabolism in man, namely, water vaporized, carbon dioxide excreted, oxygen consumed, and heat eliminated, it is necessary that the apparatus should be tested as to its accuracy for determining these four factors in experiments of short duration. In the detailed description of this apparatus results were given of a test experiment of seven and one-half hours[2] which was made by weighing an alcohol lamp at the beginning and end of the experiment, correction being made for the loss of weight during the few seconds intervening between the end of the experiment and the weighing of the lamp. Obviously, in experiments of so short a duration as one hour, a different method must be used for definitely determining the amount of alcohol burned.

In the description of the respiration calorimeter at Wesleyan University, Middletown, Conn.,[3] a form of alcohol lamp was shown to which alcohol was supplied from the outside, and the amount of alcohol was estimated with an accuracy of approximately 0.2 gm. Since the amount of alcohol used in an experiment which is designed to control the apparatus under the conditions when a man is inside is not far from 15 gm. per hour, this error is considerably over 1 per cent, and an entirely different type of lamp was finally resorted to. A hole 8 mm. internal diameter and 15 mm. deep was drilled in a small piece of brass rod 11 mm. in diameter, and the cup thus made securely attached without solder to the end of a fine copper pipe 2 mm. internal diameter and 3 mm. external diameter. This pipe was passed through an air-tight opening in the wall of the chamber near the floor, and connected on the outside with an accurately calibrated burette, on which differences of level amounting to 0.01 c.c. could be accurately read. Alcohol was then allowed to flow into this burette and through the connecting pipe into the brass cup inside, where it was absorbed by a piece of asbestos paper which served as a wick in the cup, and was there burned.

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[2] BENEDICT and CARPENTER: Carnegie Institution of Washington Publication No. 123, 1910, p. 92.

[3] ATWATER and BENEDICT: Carnegie Institution of Washington Publication No. 42, 1905, p. 100.

Considerable difficulty was at first experienced in finding an apparatus which would deliver an even flow of alcohol into the lamp. Finally an apparatus was devised which was constructed on the principle of the Mariotte flask. In this apparatus two glass tubes are inserted through a rubber stopper shellacked into the neck of a 500 c.c. bottle nearly filled with alcohol. One of these tubes, by the addition of rubber tubing, stopcock, and a capillary tube, acts as a siphon through which the alcohol is delivered. The other, which reaches nearly to the bottom of the bottle, regulates the pressure of air. When the stopcock is opened and the alcohol begins to flow out of the delivery tube, the level of the alcohol in the other tube is lowered until the tube is empty. Constant pressure is then obtained, and thus a constant delivery of the alcohol is insured. The rate of flow is regulated by a simple device for raising and lowering the point of the delivery tube.

Two of these bottles were used when making a control test, so that a second bottle could be held in readiness for interchanging at the end of each period. The bottles were adjusted to deliver 14 to 15 gm. of alcohol an hour, and this rate was maintained with the greatest regularity. It was thus possible to insure constancy in the rate of flow and to know exactly the alcohol consumption in periods of one hour.

As a result of much preliminary experimenting, which has been published elsewhere,[4] it was found that the highest grades of commercial alcohol could be used in tests of this kind. By determining the specific gravity of the alcohol used, the composition of the alcohol may be obtained from any one of the numerous excellent alcoholometric tables, and a simple computation, based on the composition of the alcohol, will give the amounts of carbon dioxide, water vapor, and heat produced, and oxygen consumed during combustion. The specific gravity

[4] ATWATER and BENEDICT: *Loc. cit.*, p. 96.

of the alcohol was determined with a Squibb pyknometer, and the alcohol used in these experiments was found to have a composition of 92.56 per cent of ethyl hydroxide by weight. Each gram of this alcohol, therefore, resulted in the formation of 1.769 gm. of carbon dioxide, 1.087 gm. of water, 6.553 calories of heat, and required for its complete oxidation 1.930 gm. of oxygen.[5] Taking into account the preformed water (0.074 gm.) and the heat used in the vaporization of water (0.687 calorie), the amounts of water and heat measured per gram of alcohol were thus 1.161 gm. and 5.866 calories respectively.

In carrying out these control tests, the apparatus was first put in running order and a preliminary test of two or three hours made. In the final test the amount of alcohol burned was weighed for each hour, and the outgoing amount of water vapor and carbon dioxide determined, as well as the amount of oxygen absorbed from hour to hour. The results of the earlier tests showed remarkable uniformity in the determination of the carbon dioxide production, oxygen consumption, and heat elimination, but there was invariably a much larger amount of water vapor in the outcoming air than could have resulted from the combustion of the alcohol.

It was very difficult to account for the large amount of water obtained in these control experiments. Apparently all of the hygroscopic material inside of the chamber was removed, including any article of wood or asbestos lumber, and even the rubber tubes of the pneumograph and stethoscope as well as many of the electric wires, and yet there was a persistent increase in the amounts of water vapor carried out from the chamber in the ventilating air current.

A series of tests was therefore made in which the regulation of the ventilating air current was such that the air entering the chamber was absolutely dry, all the moisture having been absorbed by sulphuric acid over which it had been passed. Consequently this extremely dry air absorbed moisture in the chamber and brought it out in the air current. The amounts of water vapor absorbed from the air in the chamber in the tests thus made are shown in Table III.

At the end of such a drying out there was still an appreciable amount of water vapor remaining in the air in the chamber, but since during an alcohol check experiment the air inside of the chamber is by no means absolutely dry, and under ordinary conditions there are present 5 gm.

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[5] For computation of these factors, see ATWATER and BENEDICT: *Loc. cit.*, p. 98.

of water vapor in 1500 litres of air, corresponding to a relative humidity of 19.5 per cent, it was unnecessary to provide for the removal of all the water from the chamber.

In order to test the possibility of the metal walls giving up any material amount of water, another experiment was made in which a large sheet of metal (copper), containing some 1.3 square metres, was suspended inside of the chamber on the arm of the balance. The sheet of copper was allowed to remain suspended during an eight-hour experiment, while the chamber was being dried out, and the balance, which is capable of weighing with an accuracy of 0.1 gm. indicated no appreciable loss in weight of the copper.

TABLE III.

MOISTURE REMOVED FROM THE CHAMBER BY THE VENTILATING AIR CURRENT DURING MOISTURE TESTS (QUANTITIES PER HOUR).

Date.	First period.	Second period.	Third period.	Fourth period.	Fifth period.	Sixth period.	Seventh period.
Nov.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
5	12.90	2.20	1.40	1.35	...	...	...
6[1]	2.95[1]	1.10	0.60	0.45	0.45	...	...
8	8.85	1.95	0.95	0.65	0.50	0.55	0.40
9	9.65	2.40	1.00	0.60	0.60	0.60	...
15	15.00	2.60	1.05	0.65	0.85	0.45	0.40
16[2]	1.45[2]	0.40	0.30	0.20	0.35	0.25	...

[1] Continuation of the test of November 5. The exceedingly dry air remaining in the respiration chamber over night slowly absorbed moisture from the hygroscopic material, and this moisture was measured during the first period, as may be seen from the figures.

[2] Continuation of the test of November 15. For explanation of the amount of moisture absorbed during the first period, see footnote 1.

Finally, experiments were made in which the chamber was dried out quite completely the day before, then on the morning of the experiment the window was opened, the alcohol lamp lighted, and the window closed again as soon as possible. The preliminary period of the experiment was then begun, and continued for an hour or two. Owing to the preliminary drying out process, the hygroscopic material in the chamber was comparatively free from moisture at the beginning of the experiment and thus absorbed a portion of the water given off in the combustion of the alcohol. The amount of moisture in the chamber accordingly increased somewhat and less water vapor was carried out and measured than was produced. Consequently, for the first time in experiments with this apparatus, the water found during the initial period was less than the theoretical amount, as will be seen by the results of the experiment given in Table IV. Subsequently, when moisture equilibrium had been established, the results agreed very satisfactorily with the theory.

Although all material of a supposedly hygroscopic nature was removed from the chamber, there were certain connections, particularly those leading to the resistance thermometers, that could not be removed. These consisted of copper wires covered with an insulating material commonly used for electrical connections and which doubtless absorbed an appreciable amount of moisture. The resistance thermometers, also, were wound on wooden spools, but the thorough shellacking of these spools probably prevented them from absorbing much moisture. Furthermore, while it is probable, from the results of the experiment previously referred to, that the copper wall absorbed no moisture, the white enamel paint used on the inside wall of another calorimeter has been proved to absorb a not inconsiderable amount, and this fact must be taken into consideration when using the apparatus. The amount of hygroscopic material thus remaining in the chamber, though small, is sufficient to retain water for a long time, and it was found practically impossible to make the apparatus absolutely dry in twenty-four hours.

For experiments with man this drying out process is of course impracticable, as there is always a certain relative humidity inside the chamber, and a moisture equilibrium will ultimately be established between the hygroscopic material inside the chamber and the air current. It is of no consequence, however, so far as the heat measurement is concerned, whether the water is vaporized from the surface of the copper, from the surface of rubber tubing or electric wiring, from the wood of the wooden spools of the resistance thermometers, or from the clothing, skin, or lungs of the subject, but an accurate knowledge of the total amount of water vaporized inside the chamber during a given period is a necessary factor in the measurement of the heat eliminated. This may be obtained from the analyses of the outcoming air.

TABLE IV.

ALCOHOL CHECK EXPERIMENT. — CHAIR CALORIMETER, NOV. 18, 1909.
(1-HOUR PERIODS.)

Period.	Alcohol burned.	Carbon dioxide.			Oxygen.	
		Theory.	Found.	Ratio of found to theory.	Theory.	Found.
	gm.	gm.	gm.	per cent	gm.	gm.
First	14.7	26.0	25.5	98.1	28.3	28.2
Second . . .	14.2	25.1	24.7	98.4	27.4	27.3
Third . . .	15.1	26.7	26.5	99.3	29.1	28.8
Fourth . . .	13.9	24.6	24.4	99.2	26.9	26.9
Fifth	14.6	25.9	25.9	100.0	28.3	28.7
Total . . .	72.5	128.3	127.0	99.0	140.0	139.9

TABLE V.

ALCOHOL CHECK EXPERIMENT. — BED CALORIMETER, JAN. 7, 1910.
(1-HOUR PERIODS.)

Period.	Alcohol burned.	Carbon dioxide.			Oxygen.	
		Theory.	Found.	Ratio of found to theory.	Theory.	Found.
	gm.	gm.	gm.	per cent.	gm.	gm.
First	13.2	23.4	23.5	100.4	25.5	25.4
Second . . .	13.4	23.8	23.4	98.3	26.0	26.5
Third . . .	12.6	22.2	22.3	100.5	24.3	23.5
Fourth . . .	13.8	24.4	23.9	98.0	26.6	26.5
Fifth	13.1	23.2	24.0	103.5	25.3	25.9
Sixth	13.6	24.0	23.2	96.7	26.2	25.5
Seventh . .	13.6	24.0	23.9	99.6	26.2	26.7
Total . . .	93.3	165.0	164.2	99.5	180.1	180.0

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[1] This amount does not include the water vapor for the first two periods in which obviously

TABLE IV.

Alcohol Check Experiment. — Chair Calorimeter, Nov. 18, 1909.
(1-hour Periods.)

	Water vapor.			Heat.		
Ratio of found to theory.	Theory.	Found.	Ratio of found to theory.	Theory.	Found.	Ratio of found to theory.
per cent.	gm.	gm.	per cent.	cal.	cal.	per cent.
99.7	17.0	16.7	98.2	86.1	86.1	100.0
99.6	16.5	16.4	99.4	83.3	82.8	99.4
99.0	17.5	17.5	100.0	88.5	88.9	100.5
100.0	16.2	16.2	100.0	81.7	79.8	97.7
101.4	17.0	16.9	99.4	85.9	88.1	102.6
99.9	84.2	83.7	99.4	425.5	425.7	100.1

TABLE V.

Alcohol Check Experiment. — Bed Calorimeter, Jan. 7, 1910.
(1-hour Periods.)

	Water vapor.			Heat.		
Ratio of found to theory.	Theory.	Found.	Ratio of found to theory.	Theory.	Found.	Ratio of found to theory.
per cent.	gm.	gm.	per cent.	cal.	cal.	per cent.
99.6	15.3	16.1	105.2	77.5	76.4	98.6
101.9	15.6	16.3	104.5	79.0	78.7	99.6
96.7	14.6	14.9	102.1	73.7	71.3	96.8
99.6	16.0	16.2	101.3	81.0	80.3	99.1
102.4	15.2	15.7	103.3	76.8	75.9	98.8
97.3	15.8	15.8	100.0	79.6	79.0	99.2
101.9	15.8	15.8	100.0	79.7	79.5	99.8
99.9	77.4[1]	78.4[1]	101.3	547.3	541.1	98.9

moisture equilibrium was not established. The walls of this calorimeter are painted.

The final test of the apparatus, therefore, is to burn in the chamber such small quantities of alcohol as will yield amounts of water, carbon dioxide, and heat approximating the amounts eliminated by man in periods as short as one hour when at rest inside the chamber. By means of the calorimetric and chemical features of the apparatus, measurements can be made of the total amount of heat eliminated, water vaporized, carbon dioxide produced, and oxygen consumed. The results of such experiments show that with all four factors there is a most satisfactory agreement between the amounts measured and the theoretical amounts, as may be seen by the results of the two experiments given in Tables IV and V.

To obtain duplicate results in the determination of a single element or radicle by chemical analysis frequently requires a number of determinations, and when two factors are simultaneously determined, as carbon and hydrogen in elementary organic analysis, still greater difficulty is experienced. It can therefore easily be seen that the simultaneous determination of four factors presents a problem that is rarely met with in either physical or chemical operations and heretofore never in physiological chemical operations. Indeed, the apparatus has proved as accurate as any chemical process ordinarily used in a laboratory, and it can accordingly be characterized, both calorimetrically and chemically, as an instrument of precision.

A COMPARISON OF THE DIRECT AND INDIRECT DETERMINATION OF OXYGEN CONSUMED BY MAN.

By FRANCIS G. BENEDICT.

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EVER since Sanctorius studied the insensible perspiration of man investigators have endeavored to use the insensible loss in weight for measuring the amount of water vaporized from the body. When direct determinations of the carbon dioxide production and water vaporization have been added to the measurement of the insensible loss, an attempt has also been made to determine indirectly the amount of oxygen consumed by man. Upon the completion of the great respiration chamber of Pettenkofer in Munich, a number of experiments were made in which the carbon dioxide output of man was determined with great accuracy.[1] The water vapor determination was by no means as accurate, but the authors used the results thus obtained, combined with the data regarding changes in weight of the body of the subject, to compute the oxygen consumption. Neglect to take into consideration the changes in weight of the bed and bedding led, however, to erroneous deductions from certain of these experiments.[2]

The difficulties attendant upon the accurate weighing of materials inside of a respiration chamber, and the absorption of moisture by surfaces of wood and painted metal, were later pointed out by Stohmann.[3] In more recent years Haldane[4] applied the indirect respiration chamber method to studying the oxygen consumption of small animals with considerable success. In this case it was possible to weigh not only the animal but the whole chamber in which the animal was confined, and thus the errors were minimized.

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[1] Pettenkofer and Voit: Zeitschrift für Biologie, 1866, ii, p. 459.

[2] C. and E. Voit and J. Forster: Zeitschrift für Biologie, 1875, xi, p. 126.

[3] Stohmann: Landwirthschäftliche Versuchsstationen, 1876, xix, p. 81, and p. 159.

[4] Haldane: Journal of physiology, 1892, xiii, p. 419.

When the respiration chamber at Wesleyan University was constructed, attempts were made to determine the oxygen consumption indirectly by weighing the man at the beginning and end of each experimental period. For this purpose special platform scales were installed immediately above the respiration chamber, which were connected with the weighing chair inside the chamber by a rod passing through the upper wall. This rod was made air-tight by passing it through a very light rubber diaphragm, and the whole apparatus, which has been described in detail elsewhere,[5] was so adjusted that weights could be obtained to within 5 or 10 gm.

To bring away the heat produced by the subject in this respiration chamber, cold water was passed through heat-absorbing pipes, and the variations in the humidity sometimes caused considerable condensation of moisture on the surface of the absorber system inside the apparatus. Since no provision was made for weighing this moisture which had, in a certain sense, been distilled from the body of the subject and condensed on the cold pipes, it was not taken into consideration in the ordinary course of weighing the water vaporized which had been absorbed by the air current; consequently, as an accurate measurement of the water vaporized is necessary for the indirect determination of the oxygen consumption, this method could not be used whenever such condensation occurred. Subsequently a device was attached to the heat-absorbing system so that it could be weighed with a moderate degree of accuracy, and with this appliance in use it was possible to secure an approximate idea of the oxygen consumption. As the difficulty of suspending a large heat-absorbing system on the scales was quite considerable and the limit of accuracy was not less than 5 to 10 gm., it was impossible to secure results for periods less than twenty-four hours.

Later, this respiration calorimeter was considerably modified[6] so as to provide for the direct determination of the oxygen consumption, and an extensive study of metabolism during inanition[7] was carried out. The conditions during these experiments were much simpler, as no food was ingested, and an attempt was therefore made to obtain a balance between the direct determination of the oxygen consumption and the indirect determination as computed from the changes in body weight and the weights of the gaseous and excretory products. A comparison of these direct and indirect determinations of oxygen consumption show that even in periods of twenty-four hours considerable error is liable to be made. In a seven-day fasting experiment, from March 4 to 11, 1905, variations between loss of body weight and the loss of body material as actually computed from the katabolism ranged from +15 gm. on the first day to −13 gm. on the fourth day. The average of all seven days showed an error of less than one half of a gram, — an agreement that can, from the nature of the apparatus, be no more than mere coincidence. In a series of fasting experiments with different individuals the discrepancies were from +130 gm. to −35 gm. and rarely was the agreement in any of the experiments inside of 10 gm.

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[5] Atwater and Benedict: Carnegie Institution of Washington Publication No. 42, 1905, p. 158.

[6] Atwater and Benedict: Carnegie Institution of Washington Publication No. 42, 1905. See also Benedict and Carpenter: Carnegie Institution of Washington Publication No. 123, 1910.

[7] Benedict: Carnegie Institution of Washington Publication No. 77, 1907.

The difficulties in measuring the oxygen consumption indirectly are numerous. If we assume that the computation is made according to the simple formula, $a = b + c - d$, in which a is the weight of the oxygen, b the weight of the carbon dioxide, c the weight of water vaporized, and d the loss in body weight of the subject, we can see that it is necessary to know b, c, and d, with great exactness in order to place any reliance upon the values found for a. The determination of carbon dioxide, or b, can be made with the respiration calorimeter with very great exactness. The determination of the water vaporized in the apparatus can likewise be made with great exactness under certain conditions, but it is a matter of great difficulty to secure ideal conditions for this determination.

In a respiration calorimeter recently built at the Nutrition Laboratory an attempt has been made to eliminate so far as possible all the material of a hygroscopic nature and thus secure an accurate determination of the water vaporized by the subject. The chamber is of copper, and the appurtenances inside are few and usually of a metallic nature. A most careful series of experiments, in which a piece of copper was suspended inside the chamber, showed practically no condensation of moisture on the metal, so that it is not necessary to take this factor into consideration. The hygroscopic nature of rubber tubing has also been tested thoroughly and it has been found that the tubing present in the chamber is not sufficient to yield any material amount of water to the dry air. On the other hand, the weighing chair in which the subject sits is constructed of wood, and while this is thoroughly shellacked, we have found it necessary to take into account the variations in weight of the chair, as very large amounts of water may be absorbed or given off by wood of this nature. Furthermore, for the numerous electrical connections inside the chamber well insulated wire is required, and for this insulation a fabric material has been used, which without doubt absorbs moisture. Another source of error in the measurement of the water vaporized is introduced by the resistance thermometers used in measuring the temperature of the air. These thermometers are wound on wooden spools, and although the spools are shellacked they unquestionably vary in water content.

The amount of material inside the chamber, then, that can yield water to a dry air current is quite considerable, and since there is no way of obtaining its weight it may lead to a considerable error in the determination of the water vaporized. It is possible to weigh the chair, to weigh the cushions upon which the man sits, and to weigh the body of the man, but it is impossible to note the changes in weight of the spools upon which the thermometers are wound, of the wires connecting the different thermometers, and of the different electrical connections and rubber tubes inside of the chamber.

In the preceding article tests have been cited[1] which show that considerable quantities of moisture may be removed from the chamber by passing dry air through it, even after taking out all weighable, hygroscopic material. If this method is followed before an experiment with a subject, there will then be at the beginning of the experiment very dry air inside the chamber and the hygroscopic materials will also be thoroughly well dried. When the subject begins giving up a large amount of moisture to the air, a certain amount of moisture is again absorbed and condensed by the hygroscopic materials, but an equilibrium is quickly established between the relatively dry air and the materials themselves, as is shown by the air analyses made from time to time. Under these conditions we believe that satisfactory determinations for water can be made, and that the water measured in the outcoming air is derived from the water given off by the subject himself and is not derived from any non-weighable materials inside the chamber.

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[1] BENEDICT, RICHE, and EMMES: This journal, 1910, xxvi, p. 1.

In determining the oxygen consumption by the indirect method not only must the water vaporized be accurately measured, but also the changes in body weight. To weigh a body as large as that of a 70 or 80 kg. man, continually changing in weight and losing from 0.6 to 0.7 gm. a minute, and to weigh it in a closed respiration chamber that requires an absolutely hermetical seal for the successful direct determination of the oxgyen consumption, presented problems that were overcome with great difficulty. The apparatus used in the calorimeter at Wesleyan University was by no means sensitive enough for a satisfactory determination of the body weight in periods of less than twenty-four hours, and while the platform scales which were used could, under the most advantageous circumstances, indicate definite differences in body weight with an accuracy of 2 to 5 gm., this sensitiveness could rarely be relied upon. It therefore became necessary to devise a weighing apparatus which should give more satisfactory results.

The remarkably sensitive and delicate balance and registering device of Lombard [9] could not here be used, since it would be necessary to have both the chair and the man suspended inside of a hermetically sealed box. This would involve some movable connection between the suspension rod and the walls of the chamber which could allow the rod to pass through the wall without any interchange of air. Obviously any such system reduces the sensitiveness considerably. The apparatus described by Weiss,[10] while simple and offering many advantages, was also impracticable for our use.

After repeated tests with different forms of balances a large balance was installed which was capable of suspending 100 kg. on each arm, and was sensitive with load to 1 decigram. This balance is regularly listed by scientific apparatus houses, and its sensitiveness leaves very little to be desired. One pan of the balance was removed and a suspension rod substituted. As the rod passes through the top of the respiration chamber, it traverses a long, hard rubber tube, having an internal diameter of about 4 centimetres. Between the open end of this tube and the rod a thin rubber membrane or diaphragm weighing but 1.3 gm. is attached. Under ordinary conditions and when the subject is not being weighed, the chair and the suspension rod are raised by admitting air into a penumatic cushion under the cross bar to which

[9] LOMBARD: Journal of the American Medical Association, 1906, xlvii, p. 1790.

[10] WEISS: Archives de physiologie, 1897, pp. 681–685.

the suspension rod is attached. This draws a rubber stopper tightly into the lower end of the hard rubber tube extending through the wall of the apparatus.

At the beginning of the weighing the suspension rod is lowered until the cross bar is in place and the weight can be taken by an assistant. It is impossible to secure weighings by vibration, and consequently arrangements have been made so that by raising a lever on the balance the subject can be equipoised by lead or brass weights placed in the pan on the other arm. When a sufficient number of weights have been added and exact equipoise is obtained, the time is taken to seconds. The weighings are usually made in hourly periods, which rarely vary more than forty to sixty seconds. As it is impossible to weigh the subject at any given second, correction can be made for these slight variations, and the loss in weight for the exact hour can be readily computed without introducing an error. It is believed that by this method the weighings can be made accurately to about 2 decigrams.

Even with the greatest accuracy in the determinations of carbon dioxide production and water vaporization, and with the most sensitive balance, it is still of absolute importance that the subject be very familiar with the technique. Furthermore, each time the body is weighed, the records should always include the loss in moisture and body material from the material originally weighed. Even if so small an object as a handkerchief or a lead pencil or a piece of paper is included in one weighing and not in a subsequent record and if no account is taken of the consequent loss in weight, it is clear that the omission will affect the accurate determination of the body weight. In experiments of short duration, such as five or six hours, it is particularly necessary that every precaution should be taken to make sure that the material weighed is always the same. In longer experiments of twenty-four hours [11] or more we have every reason to believe that any discrepancies appearing are due not to errors in the balance nor to errors in the determinations of the carbon dioxide and water, but to actual errors resulting from the fact that there are variations in the material weighed.

In order to obtain accurate determinations of the water vaporized and of carbon dioxide production, and exact records of the body weight, the following routine has been decided upon as the best method for conducting an experiment with this apparatus.

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[11] BENEDICT and MILNER: United States Department of Agriculture, Office of Experiment Stations Bulletin No. 175, 1907; BENEDICT: Carnegie Institution of Washington Publication No. 77, 1907.

On the day before the experiment, the apparatus is closed, and connected with the ventilating air system; the air current is then allowed to pass through the chamber for several hours. This constant supply of dry air removes most of the moisture, and the apparatus is dried to an unusual degree. At night this dry air is left in the chamber, and consequently the moisture is given up from the hygroscopic material. The next morning the current of dry air is again passed through the apparatus for an hour or more, and then the subject, who has previously eaten his breakfast, is placed inside, and the precaution is taken to seal the chamber again as rapidly as possible. An electric fan keeps the current of air in constant motion, and the subject sits in the swinging chair, which is suspended at such a height that his feet can rest on the floor of the chamber. At the moment of weighing the subject places his feet on a footrest attached to the chair and takes special precautions to see that the chair does not touch the walls of the chamber at any point and that no wires or connections are in contact with his body or with any part of the weighing apparatus. The chair is then lowered by means of the pneumatic lift, and the weighing is carried out as previously described. During the actual experimental period of weighing the ventilating air current is stopped, so that there is no undue tension on the rubber diaphragm which might vitiate the accuracy of the weighing. During the experiment the subject is cautioned to sit as quietly as possible and to spend most of the time reading. Every five minutes he presses a button which rings a bell on the outside to indicate that he is awake. At five minutes before the end of each period he is signalled and told to sit quietly with his hands in his lap and with as few muscular movements as possible until the end of the period, this being essential for the most accurate oxygen determination by the direct method.

As would be expected, but little difficulty is experienced in obtaining concordant results between the direct and indirect determinations of the oxygen consumption for periods of several hours' duration, but since the amount of oxygen consumed by man is ordinarily only about 25 gm. per hour, it is obvious that for the most accurate results very delicate manipulation is necessary to secure the direct or indirect determination of oxygen within 0.2 or 0.3 gm. Several preliminary experiments show that for periods of four or five hours' duration the direct and indirect determinations of oxygen agree perfectly. The technique has finally been so perfected that experiments of one-hour periods may also be made in which the direct and indirect determinations agree satisfactorily. The results of two such experiments are given herewith in Tables I and II. The agreement between the direct and indirect oxygen determinations in these experiments is all that could be desired, particularly when the relatively small quantities involved and the difficulties of determining the oxygen indirectly are taken into consideration, namely, the difficulties in assuming absolute values for carbon dioxide, water vapor, and losses in body weight.

TABLE I.

COMPARISON OF THE DIRECT AND INDIRECT DETERMINATIONS OF OXYGEN. METABOLISM EXPERIMENT, JAN. 28, 1910.

Period.	(a) Carbon dioxide eliminated.	(b) Water vaporized.	(c) Loss in body-wt. (Calculat'd to 60 min).	(d) Oxygen consumed (indirect determinat'n). $(a + b) - c$	(e) Oxygen consumed (direct determina'n).	(f) Ratio of indirect determination to direct determinat'n. $(d \div e)$
	gm.	gm.	gm.	gm.	gm.	per cent.
First . .	30.70	37.79	42.8	25.7	25.5	100.8
Second .	30.14	38.32	43.7	24.8	25.1	98.8
Third .	28.75	36.52	41.0	24.2	24.3	100.0
Fourth .	26.41	35.98	39.0	23.4	22.0	106.4[1]
Fifth . .	26.93	33.46	37.3	23.1	24.5	94.3[1]
Total .	142.93	182.07	203.8	121.2	121.4	99.9

[1] Obviously a compensation.

It is of interest, however, to note that in some of the unpublished experiments it was shown that in some instances where there was a disagreement between the direct and indirect determinations for short periods the evidence indicated the greater accuracy with the indirect method. This evidence is obtained from an examination of the respiratory quotients. On a given diet or when the subject had no food, the respiratory quotient followed usually a fairly regular course from hour to hour. Consequently it was possible to estimate where the discrepancies occurred, and whether they were due to the determination of the oxygen consumption by the direct or indirect methods.

This series of experiments has a particular value in that it substantiates in a striking way the accuracy of the direct determination of oxygen as made by this apparatus. A critical examination of the analytical scheme shows that when carbon dioxide is developed inside the chamber with the closed circuit of ventilation, it is practically impossible for carbon dioxide to escape from the chamber or for material amounts of carbon dioxide to enter the chamber from the room air. Consequently we have every reason to believe that the carbon dioxide determinations are unusually accurate. This is furthermore clearly shown by the evidence in the preceding paper.[12]

TABLE II.

COMPARISON OF DIRECT AND INDIRECT DETERMINATIONS OF OXYGEN.
METABOLISM EXPERIMENT, JAN. 31, 1910.

Period.	(a) Carbon dioxide eliminated.	(b) Water vaporized.	(c) Loss in body-wt. (calculated to 60 min).	(d) Oxygen consumed (indirect determinat'n). $(a + b) - c$	(e) Oxygen consumed (direct determinat'n).	(f) Ratio of indirect determination to direct determinat'n. $(d \div e)$
	gm.	gm.	gm.	gm.	gm.	per cent.
First . .	27.2	33.7	38.1	22.8	23.4	97.4
Second .	32.3	33.9	42.4	23.8	25.0	95.2
Third .	34.8	33.2	41.3	26.7	25.4	105.1
Fourth .	31.0	34.4	39.0	26.4	26.0	101.5
Fifth . .	25.5	31.2	35.7	21.0	20.9	100.5
Sixth . .	26.3	29.4	35.0	20.7	22.0	94.1
Total .	177.1	195.8	231.5	141.4	142.7	99.1

The difficulties involved in determining the amount of water vaporized have been pointed out in this and in the preceding paper. At present many experimental conditions are so adjusted as to preclude any material change in the water vaporized from the walls or from the apparatus inside the chamber. The water determinations in the alcohol check experiments are very satisfactory, and we believe that the same may hold true for experiments with man where special precautions are taken to avoid the vaporization of moisture from the material inside the chamber.

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[12] BENEDICT, RICHE, and EMMES: *Loc. cit.*

On the other hand, a slight leak in any portion of the apparatus may cause a serious disturbance in the direct determination of the oxygen consumption. Usually the conditions of pressure are such throughout different portions of the system that a leak results in a loss of air from the confined volume of the air current. Under these conditions a larger amount of oxygen would have to be admitted from the oxygen supply, and consequently there would be an apparent increase in the oxygen consumption.

With regard to the chamber itself the possibilities of a leak can be tested with great ease, and this is done before and after each experiment. The tension on the chamber is practically nothing, even the most delicate manometer indicating but a small fraction of a millimetre of water. On the other hand, in the purifying system a pressure amounting to some 20–30 mm. of mercury is required to force the air through the purifying system. If a leak occurs here, there will be a noticeable loss of air, and an excess of oxygen must be admitted. Fortunately this portion of the apparatus can also be tested with great exactness by a delicate water manometer.

It is apparent, therefore, from the results of the alcohol check experiments and particularly from the results of the determinations of oxygen by both the direct and the indirect methods, that the direct determination of oxygen with this apparatus is accurate, and that experiments on man can be made in which the direct determination of oxygen is fully substantiated by the indirect determination. Personal experience would indicate that the errors involved in the indirect determination of oxygen are such as to preclude its use under conditions that ordinarily obtain in even the most perfect forms of respiration apparatus, and that accurate determinations of the oxygen consumption of man are practicable only by means of the direct method.

While a number of years ago the close agreement between the direct and indirect determinations of oxygen shown in these experiments would have been of value as an indication that no demonstrable quantities of nitrogen were eliminated as such from the body of man, in the light of the recent brilliant research of Krogh,[13] it is unnecessary to more than point out the significance of this agreement in experiments on man as substantiating the results of Krogh when experimenting on small animals.

[13] KROGH: Skandinavisches Archiv für Physiologie, 1906, xviii, p. 364.

THE MECHANISM OF THE DEPRESSOR ACTION OF DOG'S URINE,[1] WITH SOME OBSERVATIONS ON THE ANTAGONISTIC ACTION OF ADRENALIN.

By RICHARD M. PEARCE and ARTHUR B. EISENBREY.

[From the Carnegie Laboratory of New York University.]

ELSEWHERE[2] one of us has described the depressor action of dog's urine and shown that the depressor effect of extracts of the dog's kidney is due to the urine retained in the extract, and also that in those forms of experimental acute nephritis characterized by extensive tubular destruction the depressor substance may disappear from the urine.

The present investigation was undertaken for the purpose of determining, if possible, by physiological and pharmacological methods, the mechanism of the lowered pressure. All experiments have been made on dogs under complete ether anesthesia. The blood pressure has been taken from the left femoral artery by means of a mercury manometer. Magnesium sulphate was used in most of the experiments as a wash solution for tubes and cannulas, but in the later work, at the suggestion of Dr. S. J. Meltzer, sodium citrate was substituted in order to avoid any possible depressor influence from the accidental introduction of the magnesium solution. All injections were made, with due regard to temperature and speed, into the right femoral vein. Oncometric studies of spleen, kidney, and intestine were made with the aid of gutta-percha capsules connected with bellows recorders.[3] Changes in brain volume were recorded by means of a brass cylinder penetrating into the cranial cavity, changes in a limb by the plethysmograph, and

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[1] Aided by a grant from the Rockefeller Institute for Medical Research.

[2] Pearce, R. M.: Journal of experimental medicine, 1909, xi, p. 430; *ibid.*, 1910, xii, No. 2.

[3] These were modifications of the gutta-percha spleen box first described by Schäfer. See Schäfer, E. A., and Moore, B., Journal of physiology, 1896, xx, p. 1.

those in the large veins of the abdomen by a cannula inserted into one of the common iliac veins and projecting into the inferior vena cava. The latter was connected with a water manometer. In many instances simultaneous records of changes in arterial pressure, venous pressure, and volume of spleen, kidney, and intestine were taken on the same drum, as also were various combinations of these with records of respiration and changes in the volume of the brain and leg.

The amount of urine injected was uniformly 3 c.c. The fall in pressure which results is usually from 40 to 70 mm. Hg with extremes of 20 to 90 mm. It comes on abruptly within ten to fifteen seconds after injection and returns rapidly to normal, frequently within one to two minutes and always within three to four minutes. The descent is exceedingly rapid, the tracing with drum travelling 8 cm. per minute, sometimes being almost vertical, and the lowest level is reached in five to ten seconds. At other times the curve forms an almost perfect half circle except for a slight lengthening of the return limb.

During the fall the respiration becomes slightly more rapid and deeper, but without irregularity. The pulse rate is usually not altered, but occasionally a slight quickening is seen. The amplitude of the excursion is slightly lessened during the downward course and slightly increased during the upward course of the pressure curve.

Oncometric studies show that corresponding to the fall in arterial pressure the kidney, spleen, and intestine are greatly diminished in volume, but all return rapidly to normal as the blood pressure regains its original level. In the brain and leg a very slight decrease in volume and in the vena cava, simultaneously, a barely perceptible increase in pressure are seen.

Physiological experiments in which the vagi, the cervical sympathetic nerves, the cord, and the splanchnic nerves have been cut, either singly or in various combinations, fail to prevent the fall in pressure. In these experiments the cord was cut between the first and second cervical vertebræ; and the vagi and sympathetics high in the neck. The splanchnics were cut on both sides, and in some experiments the cœliac ganglia and solar plexus were freely dissected and destroyed. After section of the cord and splanchnics the return to previous level is slow; the percentile fall in pressure is, however, always as great as the normal fall; the absolute fall, on account of the lowered pressure level, naturally being less.

As the result of such experiments, it appeared that the depressor effect must be the result of an action on the nerve endings in the vessels or on the vessel muscle itself. Physiological and pharmacological experiments undertaken for the study of this point have given interesting results.

The question of the mode of action of various substances on the neuro-muscular apparatus of blood vessels is not at all clear. The most conclusive experiments are those of Dixon,[4] and Brodie and Dixon [5] with apocodeine. This substance, when given in very large doses, paralyzes vasomotor nerve endings. "When this action is complete, pilocarpin, physostigmin, and adrenalin have no effect on the blood vessels or blood pressure, whilst barium and digitalis will still constrict the blood vessels and raise the blood pressure; the effect of digitalis is, however, considerably diminished. The former three drugs, therefore, act on nerve endings, and the latter two directly on muscle."

If these conclusions are correct, one would expect dog's urine, if it acts on the nerve endings, to cause no fall in pressure in an animal poisoned with apocodeine. If it acts on the muscle, a fall should result similar to that caused by nitrite, which, as Brodie and Dixon have shown, acts also on muscle, even when the pressure is lowered by apocodeine. We have therefore administered apocodeine (Merck) as a 1 per cent solution in an 0.85 per cent salt solution, in amounts which would be expected to abolish the adrenalin reaction. In the accomplishment of the latter we have never been entirely successful, but have been able to limit the adrenalin reaction to a rise of only a few milimetres, possibly to be explained on mechanical grounds. The following experiment is illustrative:

Dog 23, weighing 4500 gm., under ether anesthesia, received six injections of 1 per cent apocodeine during a period of eighty minutes. At the end of this time 55 c.c. had been injected, and with a pressure of 34 mm. Hg, the intravenous injection of 2 drops of adrenalin caused a rise in pressure of only 6 mm. Hg; 3 c.c. of dog's urine produced no depressor effect; 1 c.c. of 1 per cent barium chloride a rise of 28 mm.; 1 c.c. of 1 per cent nitroglycerin, a fall of 16 mm. These results are in accord with those of Dixon, and appear to demonstrate conclusively that dog's urine acts on the vasomotor nerve endings. It may also be pointed out that the fall in pressure due to apocodeine closely resembles that caused by dog's urine; they differ only in that the former is more or less permanent and the latter transient.

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[4] Dixon, W. E.: Journal of physiology, 1903, xxx, p. 97.

[5] Brodie, T. S., and Dixon, W. E.: Journal of physiology, 1904, xxx, p. 476.

We have also made pharmacological experiments which have had for their object the study of the antagonism between the action, on the one hand, of urine and, on the other, of adrenalin and of barium.

If to 3 c.c. of dog's urine are added 2 drops of adrenalin, the injection causes in an intact animal either an immediate sharp fall in pressure followed by a moderate rise, or an abrupt but slight rise followed by a fall and a slight secondary rise. In the first instance the fall in pressure is similar to that due to urine alone, but before it reaches its low level the vagus action of adrenalin is evident, and although the fall may continue, it seldom reaches the low level usually caused by urine alone and the pressure rises sharply to above the normal and there remains for some minutes. The rise of adrenalin, however, under these circumstances is never as great as that of adrenalin given alone. In the second instance both the primary and secondary rises as well as the intermediate drop are less than the effect of urine or adrenalin given alone. Rarely it has been found that a urine of marked depressor power may cause a fall in pressure entirely obscuring the effect of adrenalin. On the other hand, in animals with spinal cord, vagi, and splanchnics severed, the adrenalin rise may sometimes obscure the urine fall, though the urine alone causes a typical fall.

It is not feasible to give barium chloride and urine together because of the precipitation of the urinary salts, but 3 c.c. of urine given at the height of the rise due to barium (1 c.c. 1 per cent solution) always cause an abrupt and marked fall in pressure, with, however, an equally abrupt return to the previous level. These observations indicate not only a sharp antagonism between the action of urine on the one hand and barium and adrenalin on the other, but also that the action of urine is more powerful in the doses used, and can, during its transient action, overcome the action of the constrictor substances. These results supplement those with apocodeine, and appear to prove conclusively that the urine acts on the same structures as do adrenalin and apocodeine, that is, on the vaso-motor nerve endings. The two types of reaction caused by injecting urine and adrenalin simultaneously can be explained by assuming that in some instances the urine produces its effect on the nerve endings first and in the others the adrenalin effect occurs first.

As, however, both substances act transiently, each produces its effect after the influence of the other has been exhausted.

The action of urine during the barium rise may be explained as a temporary abolishment of the tonic contraction exerted through the nerve endings. As this is transient, the tonic condition plus the barium effect is soon resumed. Similarly may be explained the depressor effect of urine injected during the rise in pressure due to continuous electrical stimulation of the splanchnic nerves. The depressor action of dog's urine may therefore become of some practical importance in physiological and pharmacological work. As it acts in a directly opposite manner to adrenalin but on the same structures, and like adrenalin is transient in its effect and without secondary influence on the circulation, it may be used therefore to cause abrupt transient lowering of pressure as adrenalin is used to cause a similar rise in pressure.

SUMMARY.

The intravenous injection of dog's urine into the dog causes an abrupt but transient lowering of blood pressure, varying from 20 to 90 mm. Hg (usually 40 to 70), not accompanied by disturbance of heart action and with little respiratory disturbance. Section of the spinal cord, vagi, cervical sympathetic and splanchnic nerves, and destruction of cœliac ganglia and solar plexus individually or collectively do not abolish the depressor action. Physiological and pharmacological experiments based on Dixon's studies of the action of apocodeine show that when the nerve endings are so paralyzed by apocodeine that adrenalin causes little or no reaction, the urine also has no effect. Likewise, in normal animals, and those with central vasomotor influence eliminated, the action of urine is antagonistic to that of adrenalin and barium, and during the increased pressure due to electrical stimulation of the nerves of the splanchnic area, the urine causes a fall in pressure.

From these observations it is concluded that the urine lowers pressure through its paralyzing effect on the endings of the vasomotor nerves.

Dog's urine in doses of 3 c.c. given intravenously may be used in physiological and pharmacological experiments to produce an abrupt and marked but transient fall in pressure with no secondary effect, in the same way that adrenalin is used to produce a corresponding rise in pressure.

The physiological antagonism between urine and adrenalin, which evidently act on the same anatomical structures, suggests the possibility that the depressor substance of urine represents a body which previous to its elimination may have a regulatory influence on the circulation directly opposed to that of the secretion of the adrenal gland.

ON THE INTERNAL SECRETION OF THE THYROID GLAND.

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I. INTRODUCTION.

THE main purpose in this work, which is a continuation of the studies on the physiology of lymph, was to determine whether the presence of the supposed internal secretions of the thyroid parathyroid gland complex can be demonstrated in the lymph coming from these glands. The commonly accepted view to-day appears to be that the thyroid secretions reach the blood through the gland lymphatics, while there is nothing known specifically as regards the course taken by the parathyroid secretions.

1. At the very outset we are confronted by the difficulty of identification of the thyroid secretions. There appears to be considerable support for the view that the secretion is an iodized protein constituent of the gland colloid. But this theory cannot be considered proved, because of the fact that most if not all of the physiological actions of this thyroid globulin are closely paralleled by many other iodine compounds, organic and inorganic. The difference in the degree of action of the various iodine compounds may be due solely to variations in rate of absorption and of elimination of the iodine in the different combinations. These tests for thyroid secretions may therefore simply be tests for iodine. Our present experiments were, however, undertaken mainly on the assumption that the thyroid globulin is the physiologically important secretion of the gland.

2. The contents of the gland follicles are, of course, a product of the gland cells; and in that sense the colloid, when present, is obviously an internal secretion, or a degeneration product of the gland. But it does not follow from this that the colloid or any of the constituents of

the colloid constitute the physiologically important secretions. So far as we know the colloid has never been isolated from the gland cells in sufficient quantity for chemical and physiological tests. Nevertheless, attempts have been made to determine the colloid factor by comparative chemical and physiological tests of glands rich in colloid and glands free from visible colloid, but at most the difference obtained has been only one of degree, and we must, moreover, reckon with possible variations in the cell content of glands under different physiological conditions. Some work of Oswald[1] seemed to show that the iodized protein is confined mainly, if not wholly, to the colloid in the follicles. He states that no iodine can be detected in calf thyroids free from colloid, and that the iodine percentage is proportional to the quantity of the colloid in the gland. He admits, however, that thyroid globulin can be obtained from iodine free glands. This attempt of Oswald to confine the iodized thyroid protein to the colloid has not been supported by the later workers. Thus Marine and Williams[2] and Marine and Lenhart[3] find that the iodine percentage is greater in normal glands than in colloid goitres rich in colloid, and that iodine is present in glands — but in smaller percentage — free from colloid. It is obvious that the iodine, if present in the follicle colloid, must pass through the gland cells to get there, and that the iodine must therefore be present in the cells themselves. We might recall in this connection the supposed finding of colloid within the cells themselves (Langendorff, Hürthle, and others), although these observations are strongly questioned by the more recent works of Lewandowsky, Lobenhoffer, and others. In brief, the proofs of the hypothesis that the follicle colloid constitutes or contains the physiologically important secretion of the gland are still lacking.

3. But assuming that the colloid constitutes or contains the physiologically important secretion of the gland, the common view that these secretions reach the blood by way of the lymph stream rather than directly through the blood capillaries appears to be founded on the following observations:

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[1] Oswald: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 265; xxxii, p. 14; Beiträge zur physiologischen Chemie, 1902, ii, p. 545.

[2] Marine and Williams: Archives of internal medicine, 1908, i, p. 358.

[3] Marine and Lenhart: Archives of internal medicine, 1909, iii, p. 66; iv, p. 440.

(1) The early histological studies, especially of Baber,[4] Biondi,[5] Langendorff,[6] and Hürthle,[7] appear to have established the fact that the secretory acini in the thyroid are surrounded by a rich network of lymph spaces or capillaries, and that these lymph spaces and even the larger collecting channels are frequently filled with a colloidal substance, apparently identical with that within the follicles of the gland. That the two colloids were similar in structure and composition seemed to be indicated by their reactions to stains and other reagents. Two methods of passage of the intrafollicular colloid into the lymph capillaries have been suggested. According to Biondi and Langendorff the cells in certain regions of the acini become progressively flattened, and then they undergo colloidal degeneration *in toto*, with the result that the follicle space is fused with the lymph space. Langendorff is not certain that this is the only mechanism of transfer of the follicle colloid into the lymphatics. (2) Hürthle accepts this method of colloidal degeneration and rupture of the walls of the follicles, but he endeavors in addition to demonstrate the existence of minute canals between the follicular cells by injection methods; and he appears to look upon these canaliculi as the ordinary avenues of the colloid transfer.

We think that but little importance can be placed on the injection experiments, as slight tension will make passages through delicate but continuous membranes. Furthermore, the fusion of follicles and lymph spaces may be artifacts, pure and simple, especially in view of the fact that Hürthle describes as of frequent occurrence the presence of erythrocytes and leucocytes within the follicles. The latter condition is certainly due to rupture and displacements. Lewandowsky,[8] working with microchemical methods on the glands of a number of mammals, rejects the view that the colloid passes from the follicles into the lymph spaces. He holds that the colloid state is due to a gradual change in the condition of the protein solution after it has left the secreting cells, and that the secretion may similarly be transformed into colloid in the lymph spaces. Lobenhoffer[9] holds that the acidophile granules in the thyroid cells differ from the colloid microchemically, but on being extended from the cells they are gradually converted to colloid.

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[4] Baber: Philosophical transactions, 1881, clxxii, B, p. 557.

[5] Biondi: Berliner klinische Wochenschrift, 1888 (No. 47).

[6] Langendorff: Archiv für Physiologie, 1889, Suppl. p. 219.

[7] Hürthle: Archiv für die gesammte Physiologie, 1894, lvi, p. 1.

[8] Lewandowsky: Internationale Beiträge zur inneren Medizin, 1902, ii, p. 387.

[9] Lobenhoffer: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1909, xx, p. 650.

The colloid, once in the tissue spaces, may enter the blood directly through the capillary wall, or indirectly by being absorbed by the lymphatic capillaries. Horne[10] described colloid in the veins of goitrous glands, but Hürthle's criticism renders this observation doubtful.

That the colloid may, however, under certain conditions leave the follicles — probably by resolution and absorption into the gland cells — is evident from the fact that normal gland as well as colloid goitres may pass into active thyroid hyperplasia free from visible colloid.

II. Lymph Production in the Thyroid.

1. **Lymph production in normal thyroids.** — Our work has been done on dogs, cats, and foxes, the major part being confined to the dog. In these animals the main lymphatics leave the upper pole of the thyroid gland in close proximity to the superior thyroid veins, and join the main neck lymphatics on either side. Smaller lymphatics may leave the gland at the inferior pole — and more rarely in the middle region — to join the neck lymphatics further down. This relation of the thyroid lymphatics applies also to all forms of goitres in these animals, the only difference being the great enlargement of the lymphatics in the latter conditions. In consequence of this relation of the thyroid lymphatics to the main neck lymphatics all the thyroid lymph secreted for a certain period may be collected or eliminated from the animal by placing cannulas in the main neck lymphatics below the point of entrance of the thyroid branches. This is of importance because of the fact that in all normal thyroids the gland lymphatics themselves are too small for direct experimentation.

In the case of normal thyroids of these mammals the gland lymphatics are very small, smaller than from salivary glands of corresponding size. The rate of lymph production in such normal glands may be estimated by placing a cannula in the main neck trunk and ligating all the branches above the thyroid. This procedure practically stops the flow of lymph even when the thyroids are massaged directly. It is therefore evident that the *rate of lymph production in the normal thyroids is very slow, probably not exceeding* 2 c.c. *in twenty-four hours.*

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[10] Horne: Journal of anatomy and physiology, 1893, xxvii, p. 161.

There is a very obvious corollary to the above fact, namely, that unless the future brings forth chemical or biological tests of the thyroid secretions of very much greater delicacy than our present supposed tests, there is little hope of demonstrating the presence of these secretions in the lymph of normal glands by direct experiments, even if the secretions reach the blood by that route.

2. **Lymph production in goitres.** — (1) This work was confined to dogs, as we have never observed goitres either in cats or foxes. It is well known that goitre in dogs is endemic in the region of the Great Lakes of the United States. Marine and Williams estimate percentage of goitres in dogs as high as 90 in the city of Cleveland, Ohio. We have made no accurate percentage determination, but if the rigid standard adopted by these workers be a criterion for normal thyroids, the proportion of goitre in Chicago dogs would probably be as high as in Cleveland. Certainly over 50 per cent of the dogs brought to our laboratory in the course of a year have distinct or palpable enlargement of the thyroids.

Marine and his co-workers have made extensive histological and chemical studies of these dog goitres. They arrange them under three types, — colloid, active hyperplasia, and mixed, — and conclude that there is no essential difference in histological structure and iodine content of these types of goitres in dog and man. We wish to emphasize this conclusion of Marine, in view of the symptoms or rather absence of symptoms in these goitrous dogs.

(2) If the histological structure and the iodine content of the thyroids are true indices of the physiological states of the gland, and if the physiological states of the glands are causally — direct or indirect — related to the symptoms of cretinism, myxedema, and exophthalmic goitre, we ought to find these symptoms in the goitrous dogs. The striking fact is, however, "that these goitrous dogs are to all appearance normal" (Marine), with the exception of the goitre.

The absence of cretins among the dogs may be due to the failure of survival. Marine records cretin symptoms in five pups with corresponding thyroid changes, the iodine test being negative. We have so far met with only two dogs (both young) that in stunted growth in form ("pot-belly") and intelligence suggested cretinism. The thyroids were enlarged, but the iodine tests in both dogs were positive. The following extract from the protocol of one of these dogs may be cited: Weight, 4.5 kg.; pulse, 120–130, irregular; respiration, 20–24; weight of thyroids, fresh, 108 gm., dried, 27 gm.; total iodine, 0.4 mgm. Dog depressed and stupid in appearance. No indication of myxedema.

Symptoms of myxedema ought to appear in at least a certain percentage of the colloid goitres, but we have never observed any goitre dog that even by a considerable stretch of imagination could be called myxedematous. There is no obvious loss of hair, dry skin, depression of the intelligence, or alteration of the connective tissue.

The search for the symptoms of exophthalmic goitre is equally futile. The active hyperplasia dogs as defined by Marine exhibit neither tachycardia, emaciation, nor nervousness. To be sure, among the several thousand dogs observed in the past three years, cases of emaciation and irregular pulse and nervousness have been noted, but these appear to be as frequent in dogs without thyroid enlargement as in the hyperplasia subjects.

When the thyroid enlargement is very great, 500 to 800 gm., we have frequently noted depression; and such animals usually die easily under anæsthesia. But this may be due in part to mechanical interference with circulation and respiration. And post mortem examination of these animals has in most cases revealed additional pathological conditions, such as aneurisms, dilation of the heart, tumors, cirrhosis of the liver, arterial sclerosis, etc., which undoubtedly contributed to the symptoms of weakness and depression.

Moreover, there is no relation of the thyroid hyperplasia to sex, as is the case in exophthalmic goitre in man. In brief, there is no evidence in goitrous dogs of either myxedema or exophthalmic goitre, apart from the thyroid enlargement. We have not spoken of the exophthalmus, as the relation of this symptom to Graves disease is obscure. And in our judgment it is very difficult to decide on this phenomenon in dogs, as the prominence of the eyeballs varies in different breeds of dogs. But we have not been able to connect any type of thyroid enlargement with exophthalmus. It seems to us, therefore, that unless future work on these goitre dogs with strength tests and in metabolism shall establish some points of contact with myxedema and exophthalmic goitre in man besides that of similarity in thyroid structure, the identity of these goitre states in man and dog is problematical.

(3) Lymph production in goitres is greatly increased over that in the normal gland. This is true for all the types of thyroid hyperplasia

found in dogs. Marine and Williams state that the size of the gland lymphatics decreases as the gland passes from active hyperplasia to the colloid state. There is no clear evidence of this on the side of rate or volume of lymph output from the gland. It is well known that the volume output of lymph from the head and neck region is variable, depending among other things on such uncontrollable factors as age, state of nutrition, etc. And allowing for such factors the lymph production in goitres seems to be directly proportional to the size of the gland, irrespective of the type of goitre. This is also true for cystic or hematomatous goitres as well as for thyroid tumors (carcinoma).

In our experiments the animal was kept under light ether anæsthesia, administered by intubation. It is our experience that lymph production in the head and neck region is diminished by anæsthesia proportional to its degree. It is therefore probable that the lymph flow from the glands as observed by us was less than the normal.

The lymph issues from the goitres spontaneously, but the rate of flow is greatly augmented by massage of the gland. When thus directly massaged goitres weighing from 100 to 500 gm. (fresh tissue) will yield 40 to 60 c.c. of lymph in the course of two hours. Without massage only about half of that quantity is obtained. The largest quantity yielded by any goitre in our experience was obtained from a carcinomatous thyroid, one lobe of which yielded 60 c.c. in ninety minutes.

The general character of the goitre lymph is the same as that of the common neck lymph, with the exception that it is poorer in lymphocytes. In some cases it appeared to be richer in fibrinogen. In hematomatous glands the lymph has often a brownish color due to admixture of the hematomatous fluid. This is in all probability an artifact, due to rupture of membranes by massage or during the struggle attendant upon anæsthesia. Prolonged massage of the gland causes the appearance of erythrocytes in the lymph; but the same is true of massage of other organs.

While no exact figures can be given for the quantity of lymph produced in twenty-four hours in normal and in goitrous glands, it is a safe estimate that the latter exceeds the former ten to a hundred times, depending on the degree of hyperplasia. In very large goitres of average-sized dogs (12 kg.) *more than 200 c.c. of lymph is emptied into circulation from the thyroids in the course of twenty-four hours.*

The fact that in general the lymph production in the head and neck region is greater in young than in old animals suggests that it is in some ways correlated with growth. The copious lymph production in the carcinomatous thyroids points to the same thing. We ought on this basis to have a greater lymph production in active hyperplasia associated with cell division and rapid growth than in the colloid or relatively quiescent goitres, but if such a difference exists it is not great enough to be demonstrated by the present methods.

(4) Is there any relation between the percentage of iodine in the gland and the volume output of lymph from the gland? It is still an open question to what extent the rate of lymph production is related to the degree of physiological activity of an organ. The theory of Asher has been shown to be untenable for the salivary glands, but it may be tenable for organs having no external secretion, although we know of no experiments directly supporting the theory in the case of muscle, nervous tissue, and the ductless glands. If it is applicable, we should have to conclude that all types of thyroid hyperplasia in the dog are accompanied by increased physiological activity of the gland. We were primarily interested in the relation between the lymph production and the iodine content of the gland in relation to the possible application of Asher's theory.

Assuming that the iodine is an element in the thyroid secretion, the significance of greater or less iodine in the gland itself is still a matter of conjecture. The higher percentage of iodine may mean a storage of the element in the gland in some form analogous to secretion granules in other glands and thus signify relative quiescence of the gland. On this interpretation paucity of iodine would mean greater physiological activity. But the very opposite interpretations might be made with equal or possibly greater degree of probability.

Iodine determinations were not made in all the goitres from which the lymph was collected, but a sufficient number of determinations were made to show that *there is no correlation between iodine content and lymph output.* The correlation is with the size of the gland and not with the iodine content. Thus there is just as copious lymph flow in goitres having only a trace of or no demonstrable iodine as in glands of equal size having moderate and up to great quantities of iodine. For example, the lymph flow in a carcinomatous thyroid weighing 340 gm. and containing about 33 mgm. iodine was if anything a little less than in a similar thyroid weighing 326 gm. but containing no iodine. Similar correspondence was noted in four cases of simple hyperplasia with traces of iodine and with relatively high iodine content respectively. It might be emphasized again that small variations in the lymph output are of no significance because of the many variable factors. In fact, by varying the massage or the degree of anæsthesia one can get rates of lymph flow to suit any theory that might be proposed.

So far, then, the significance of the augmented lymph output in thyroid hyperplasia and thyroid tumors is not apparent. The correlation may be simply that of vascularity in the gland. But the kidneys or the spleen are also very vascular organs. And it is indeed estimated that the rate of blood flow through the kidney per bulk of organ is greater than that through any other organ of the body. Yet in dog goitres of the size of a kidney or the spleen the lymph production is many times greater than in either kidney or spleen. Hence the correlation with the blood vessels seems insufficient, but it is no doubt one of the factors. Growth is in all probability another factor. Whether the special physiological activity of production of the internal secretions (and possibly detoxication processes) also constitutes a factor remains to be determined.

III. TESTS FOR THYROID SECRETIONS IN THYROID LYMPH.

1. **The test for iodine.** — The Baumann method was employed nickel crucibles being used for incineration and fusion, as recommended by Oswald. Lymph from the goitres of ten dogs were tested in quantities from 10 to 200 c.c., the average quantity for each test being 55 c.c. The 200 c.c. sample was collected from three dogs. *The tests were uniformly negative.*

Iodine tests were made on most of the goitres which yielded the lymph tested for iodine. In three no iodine was found in the glands themselves. In four the test was positive, one of these four containing 15 mgm. of iodine. The negative results do not prove, of course, that no iodine compounds are present in the thyroid lymph. They simply show that if such compounds are present, they are present in too minute traces to be detected by our present chemical tests in the quantity of lymph at our disposal. It should be remembered, however, that the quantity of thyroid lymph used in most of the tests was probably as great or greater than that secreted by the normal gland in the course of a whole week. And the active massage of the gland would seem to favor the passage of our hypothetical iodized protein or colloid into the lymph, if they pass by that route at all.

2. **Intravenous injection of thyroid lymph in dogs under anæsthesia.** — In these experiments we looked for effects on the circulation similar to those that have been described as following the intravenous injection of thyroid extract and thyro-globulin. This work was begun by Oliver and Schäfer,[11] and extended principally by von Cyon,[12] von Cyon and Oswald,[13] and von Fürth and Schwartz.[14] The earlier work seemed to show that thyroid extracts and the thyro-iodine lower the blood pressure, mainly by peripheral action. The injection may also retard and strengthen the heart beat. It is not necessary to refer in detail to von Cyon's theory of thyroid action on the heart nerves. Now, in the light of all the recent work on the action of tissue extracts on the circulation, it is obvious that this lowering of the blood pressure by thyroid preparations is nothing specific. And von Fürth and Schwarz conclude that thyro-iodine or thyroid extracts have no direct or specific effect on the circulation, but that the fall in the blood pressure on intravenous injections is due to traces of cholin in the preparations. They found, moreover, that subcutaneous injections of thyro-iodine (0.22 to 2.25 mgm. I) daily for ten-day periods bring on a temporary tachycardia, but the heart returns to normal before the end of the period of injections. The same results are produced by some artificially iodized proteins and by potassium iodide.

It is therefore evident that the supposed direct and specific action on the heart and the blood vessels of the thyroid secretion (thyro-globulin) is, to say the least, extremely problematical. But this phase of our investigation was under way before the publication of the work of von Fürth and Schwarz. And as the results seem to indicate some pathological action of lymph and blood in goitrous dogs they may be briefly recorded.

Intravenous injections of thyroid lymph from six goitrous dogs in quantities from 25 c.c. to 50 c.c., thoracic lymph from two goitrous dogs (35 c.c. each), and defibrinated blood from three goitrous dogs (50 c.c. each) were made in a corresponding number of normal dogs under anæsthesia and prepared for blood-pressure recording. Prior to the injection of the foreign fluids quantities of blood equal to those to be injected were withdrawn from the animal, so as not to alter the total quantity in the circulation. The injections were gradual, being completed in two minutes. The observations were continued for two to three hours after the injections. The goitres of the dogs yielding the fluids injected varied in weight from 100 gm. to 600 gm. (fresh tissue).

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[11] Oliver and Schäfer: Journal of physiology, 1895, xxiii, p. 277.

[12] von Cyon: Archiv für die gesammte Physiologie, 1895, lxx, p. 126; 1898, lxxiii, p. 42.

[13] von Cyon and Oswald: *Ibid.*, 1901, lxxxiii, p. 199.

[14] von Fürth and Schwarz: *Ibid.*, 1908, cxxiv, pp. 113, 261.

(1) There is no immediate effect on the heart or on the blood pressure in the case of either the lymphs or the blood.

(2) In two out of the six cases of thyroid lymph there was no discernible effect either on the heart or the blood pressure. The observations continued for three hours, and towards the end there was a slight gradual fall in the pressure, and some weakening of heart beat, but that was in all probability due to the anæsthesia (ether).

(3) In the remaining experiments the injections were followed by a gradual fall of the blood pressure and some acceleration, weakening, and irregularity of the heart. These symptoms appeared within ten to twenty-five minutes after the injection and in no case did the pulse or the blood pressure return to normal. The cardiac acceleration was usually more marked than the fall in the blood pressure. In two cases Traube-Hering blood pressure waves appeared intermittently, but it is known that these may follow the intravenous injections of neck lymph from normal dogs (Asher, Carlson).

While there is nothing in these results that points specifically to thyroid action, they seem to show that in some cases the blood and the lymph of dogs with very large thyroid hyperplasia contain substances that act injuriously on the heart and the circulation when introduced intravenously into normal dogs. Transfusion experiments from goitrous dogs to normal dogs might shed further light on these phenomena.

3. **Intravenous injections of thyroid lymph into normal dogs not under anæsthesia.** — The symptoms looked for in these series were those of "hyperthyroidism," particularly the tachycardia and nervous excitability. We were not in position to study the respiratory exchange and the nitrogen excretion. It appears to be well established that when given in sufficient quantity thyroid or thyro-iodine produces, temporarily at least, tachycardia, nervous excitability, and increased metabolism

(CO_2, and possibly nitrogen, output). This is true whether the thyroid administration is made by mouth, hypodermically, or intravenously. But relatively large quantities of thyroid are required to produce these symptoms. And according to the observations of von Fürth and Schwarz on dogs, the tachycardia is only temporary even on continued thyroid administration.

In six of the experiments the procedure was as follows: Small dogs with relatively normal thyroids were selected, and their temperature and pulse recorded; they were then lightly etherized to admit of the intravenous injection without pain or struggling. Defibrinated goitre lymph in quantities from 25 c.c. to 60 c.c. was injected in one of the ear veins, and the pulse, temperature, and general behavior of the animals noted for twelve to twenty-four hours. Parallel experiments were in most cases made with corresponding quantities of blood from the goitre dogs yielding the lymph. No injections were made of lymph from the thoracic duct. In four experiments the lymphs were injected without anæsthesia. Our results were as follows:

(1) There appears to be no qualitative or quantitative difference in the symptoms produced by the blood and the thyroid lymph from the same animal.

(2) In every case there was a rise of body temperature of from 2° to 4° C., lasting from four to twenty hours. The maximum temperature elevation is reached within an hour after the injection; the return to normal is more gradual.

(3) Some irregularity of the pulse occurs, and this is usually accompanied by acceleration of the heart. In two cases the cardiac irregularity was accompanied by a slower pulse.

(4) Three of the dogs showed slight tremors, the remaining exhibiting no untoward symptoms, despite the elevated temperature.

In no case did any of the above symptoms persist for more than twenty hours. In so far as these symptoms are due to substances in the injected fluids, it is obvious that these are present both in the blood and in the thyroid lymph in these goitre dogs. The fluids injected were not absolutely sterile, but the rise in temperature appears to us to be too sudden and of too short a duration to be due to an infection. The heart changes suggest thyroid tachycardia, but these effects on the heart may be secondary to the rise in body temperature. And thyroid administration is not known to produce fever so far as we are acquainted with the literature.

Control experiments with blood and lymph from normal dogs were not made. But in another research one of us had occasion to make a great number of transfusions of blood from one normal dog into another, and this is invariably followed by some elevation of the body temperature. In those experiments, however, the animals were anæsthetized, the carotid artery and the jugular vein isolated, etc., so that it is not possible to determine what part is played by the foreign blood in the temporary disturbance of the heat regulation mechanisms.

4. **The aceto-nitrile test of Hunt.** — (1) Hunt[15] and Hunt and Seidell[16] have shown that mice fed on thyroid preparations exhibit an increased resistance to aceto-nitrile, and that the efficacy of the thyroid in producing this resistance is, on the whole, proportional to its iodine content. Thyroid-fed rats exhibit, on the other hand, a decreased resistance to the poison. The importance of this discovery in relation to the physiologically important thyroid secretions seems to be called in question by the fact, as recorded by Hunt, that other iodine compounds produce similar immunity or reduced immunity, but to a less degree. And later works of Hunt seem to show that the resistance to aceto-nitrile in mice may be greatly altered by variations in diet quite apart from thyroid or iodine preparations, and that preparations from other organs, when fed, give similar immunity but to a less degree. Very small quantities of thyroid suffice to give the immunity to mice, and Hunt estimates that the test is forty times more delicate than the chemical test for iodine in the thyroid.

Because of this great sensitiveness of the test we decided to apply it to thyroid lymph, although we recognized that it is still an open question whether the test is one for iodine or for the physiologically important thyroid secretions.

(2) We carried out seven groups of experiments with thyroid feeding, simply repeating Hunt's procedure. Each group consisted of ten mice, five thyroid-fed and five controls. The thyroid-fed mice were given a mixture of cracker dust and thyroid tablets (Armour's) varying from one fifteenth to one tenth of a grain of desiccated thyroid per day for six to eight days. The controls were fed on crackers or crackers mixed with some serum albumin. In four of these groups *the aceto-nitrile in-*

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[15] Hunt: Journal of biological chemistry, 1905, l, p. 13; Journal of American Medical Association, 1907, xlix, pp. 240, 1393.

[16] Hunt and Seidell: Hygienic Laboratory, Bulletin 47, Washington, 1909.

jections confirmed Hunt's results, the thyroid-fed mice recovering from doses one-half to two and one-half times larger than those that killed the controls. In the case of the three remaining experiments no increased resistance was shown by the fed groups, but the individual variations in the resistance were so considerable that it does not seem strictly fair to designate them as negative. They are non-committal.

In general, then, the results of Hunt, and Hunt and Seidell are confirmed; but we were not able to secure as marked a difference in the immunity of the fed and the control groups as that recorded by these observers (13 to 20 times).

(3) As a preliminary to the tests on thyroid lymph we fed three groups of mice on blood from three normal dogs. For eight to ten days each mouse received 1 c.c. of the blood mixed with crackers. The results of the aceto-nitrile injections were negative. This is in line with Hunt's findings in a number of other mammals, including man. Feeding mice normal blood, at least in the animals so far attempted, does not affect the aceto-nitrile immunity. It is therefore evident that, assuming the test is one of thyroid secretion, it is not delicate enough to detect these secretions in the blood of normal mammals.

(4) Six groups of tests were made on lymph from colloid and hyperplasia goitres, 1 c.c. of lymph mixed with crackers being fed to each mouse for six to eight days. The controls were given crackers only. No controls were made by feeding the blood or the lymph from the thoracic duct from the same dogs. The results of the aceto-nitrile injections were uniformly negative, with the exception that in two of the groups the individual variations of the mice, fed as well as control, were so considerable that much importance cannot be attached to them one way or the other. The remaining four experiments were, however, clearly negative. We confess we were both surprised and disappointed in the negative outcome of these tests, for even if the test is not one of thyroid secretion, it appears to be at least an exceedingly delicate test for iodine, and it would seem that even iodine is not present in thyroid lymph in demonstrable quantities.

The thyroid secretions may not be present in the lymph, or the quantities of lymph used in feeding were not sufficient, or, lastly, the Hunt test is not a test of the secretions. And we stopped our work on this phase until this last alternative could be further tested. Hunt himself found that the test is negative on the blood of guinea pigs, even after prolonged and excessive thyroid administration, when we ought to expect increased quantities of thyroid secretions or thyro-iodine in the blood. But assuming the correctness of the Moebius theory of the relation of the thyroid secretion to the symptoms of exophthalmic goitre, Hunt has tested the blood from three cases of exophthalmic goitre in man, the results in one case being negative, in the other two positive. The iodine test made on 150 c.c. of blood that gave the greatest immunity by the feeding test on mice was negative. We have had the opportunity of making one feeding test on blood from one typical exophthalmic case (woman, age 22) through the courtesy of Dr. A. Werelius. The tachycardia and excitability were pronounced, the thyroid greatly enlarged, and the emaciation extreme. The blood was secured post mortem, and fed to six mice as in previous experiments. The aceto-nitrile injections gave clear-cut negative results. But even positive results from the test on blood in Graves disease would not necessarily prove the presence of thyroid secretion, as other organs give some immunity, and there is ample evidence in exophthalmic goitre of altered activity of other organs besides the thyroid. For example, recent work indicates even an excess of adrenalin in the blood in such patients.

Inasmuch as the Hunt test is negative with normal mammalian blood, there is no hope of gaining further knowledge by comparing the effects of feeding normal blood and blood from completely thyroidectomized animals, despite the fact that the thyroid secretions in the blood of thyroidectomized animals must gradually diminish. But it seems to us that the test can and should be tested in the following ways: Feeding experiments with blood of animals in "experimental hyperthyroidism," if such a condition can actually be produced experimentally, ought to produce the immunity. And, secondly, diminishing the quantity of the thyroid secretions in the blood of mice and rats ought to decrease the natural immunity in the former and increase it in the latter. If these two lines of work should yield only negative results, the value of the Hunt test for the thyroid secretions becomes very doubtful. This work is now being carried out in our laboratory by Messrs. Lussky and Olds. And it may be added in a preliminary way that the thyroidectomy experiments both in mice and rats have so far given uniformly negative results.

5. **The effects of complete elimination of neck and thyroid lymph for forty-eight hours in normal foxes.** — (1) If the internal secretions of

the thyroid complex reach the blood by way of the lymphatic lymph, it ought to be possible to produce the earlier and primary symptoms of complete thyroidectomy by complete elimination of the neck lymph, provided this can be done for sufficiently long periods. Ligation of the neck lymphatics will not suffice, because under those conditions there is either a stasis in the lymph production in the region involved or else the lymph is absorbed by the blood vessels of the lymphatics. One of us, working with Dr. Greer on another problem, had occasion to ligate the neck lymphatics on both sides in five dogs. In three animals there was not even a temporary edema of the head region; one exhibited slight edema for two days, followed by complete recovery, while the fifth dog developed extreme edema of neck, face, and head, and this led in a few days to necrosis of the gums and lips, and infection of the lymph glands.

After some experimentation we concluded that the most feasible way to effect complete elimination of the neck lymph is to isolate and ligate the common lymphatic trunks as low down in the neck as possible, and then bring the cephalic ends to the exterior through a slit in the skin, fix them there by skin sutures, and make a longitudinal slit in the lymph vessels to allow free escape of the lymph on the surface of the neck. The essential point is to isolate the lymphatic trunks so that there is no occlusion of them by pressure or muscular contraction, and in fixing them to the surfaces of the skin care must be taken that they are not put on stretch, because in that case they will be ruptured by the bending of the neck. The neck may be dressed lightly with absorbent cotton. These lymph fistulæ, when successful, will remain open and allow free escape of the neck lymph from thirty-six to forty-eight hours. There is little or no trouble from coagulation. But the portion of the lymphatics outside seems to disintegrate gradually, with the result that their attachment to the skin is broken and the central portion disappears through the skin wound. The lymph may continue to escape through the skin wound for a day or more after this has happened.

This method of attack would not have occurred to any one as possible, on the basis of what is known of the symptoms of complete thyroidectomy in dogs and cats, as in these animals it is exceptional that symptoms appear within forty-eight hours after operation, and we have seen that it is practically impossible to effect complete lymph elimination for a longer period. But the results of Vincent and Jolly [17] on foxes gave us hope of applying our method in these mammals. Four out of the five foxes completely thyroidectomized by Vincent and Jolly died in tremors and convulsions within ten to twenty-four hours after the operation, while one lived for a longer time. It is obvious that in animals with such rapid onset of the symptoms crucial experiments can be made with the method under consideration.

In the fall of 1908 we secured two full-grown foxes in good condition, one of which was lost accidentally, and in the spring of 1909 we were fortunate in securing two litters of nine each. These were raised until October, when they were of sufficient size for the work.

(2) We record five successful experiments with the lymph eliminated as above. In one of the experiments cannulas were placed in the neck lymphatics and the lymph actually collected under continuous massage of the neck from 12.30 to 9 P. M., when the lymphatics were fixed to the skin in the manner described. Our results were uniformly negative, all the animals remaining to all appearance normal throughout. Under ordinary conditions we would not have rested with only five successful experiments, but as soon as we discovered that our thyroidectomized foxes did not respond in the way described by Vincent and Jolly, this phase of the work was given up as futile. These results are not only negative, but they are of no value even as negative results, except possibly in emphasizing the variability of the hypothyroid symptoms in the same species.

(3) The effect of complete thyroidectomy in foxes. The foxes used in the lymph elimination series were allowed to heal from the operations and were subsequently thyroid-parathyroidectomized. Others were completely thyroidectomized in the course of another research on the liver, so that to date the record is twelve. The results are best presented by the following brief protocols:

PROCTOCOL: COMPLETE THYROIDECTOMY IN FOXES.

I. MALE OVER ONE YEAR OLD.

1908

Nov. 2. Glands removed; good recovery.

Nov. 3–4. No symptoms; good appetite.

Nov. 5–6. Rather depressed and lethargic; no tremors; does not eat.

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[17] VINCENT and JOLLY: Journal of physiology, 1905, xxxii, p. 65.

Nov. 7. Violent tremors with intermittent convulsions from 9 A. M. to 2 P. M.; salivation, rapid breathing. After 2 P. M. quiescent but weak.

Nov. 8. Weak; no other symptoms.

Nov. 9–Dec. 15. Fox normal in every way; gained in weight.

Dec. 16. Fox killed and post-mortem search made for accessory thyroids and parathyroids in neck and chest. None found. Thirteen glandules sectioned as possibly containing thyroid tissue, but they all proved to be lymph glandules.

II. FEMALE, OVER ONE YEAR OLD.

1909

Oct. 28. Glands removed; good recovery.

Oct. 29. Normal, but does not eat.

Oct. 30. Normal; eats.

Nov. 1 to date (Jan. 25). Fox normal to all appearance. No myxedema; cachexia, or emaciation.

III. FEMALE, LESS THAN ONE YEAR OLD.

Oct. 28. Glands removed; good recovery.

Oct. 29. Normal, but does not eat.

Oct. 30. Salivation, rapid breathing, strong tremors and tetany from 7 A. M. to 2 P. M.; quiescent but weak during the rest of the afternoon.

Nov. 1. Normal, but rather weak; eats; no sign of tremors or salivation during the day.

Nov. 2. Restless; slight tremors; dyspnœa; does not eat.

Nov. 3. Some depression, but no tremors or salivation until 4 P. M.; does not eat. At 4 P. M. spasms appeared and continued unabated as long as observed (7 P. M.).

Nov. 4. Found dead at 7 A. M. Post-mortem examination revealed no parathyroids nor accessory thyroids.

IV. MALE, LESS THAN ONE YEAR OLD.

Nov. 1. Glands removed; good recovery.

Nov. 2. Normal; eats.

Nov. 3. Slight tremors and spasms from 7 to 11 A. M.; normal during the afternoon; does not eat.

Nov. 4. Depression and slight spasms. Killed for liver perfusion.

V. MALE, LESS THAN ONE YEAR OLD.

Nov. 12. Glands removed; good recovery.

Nov. 13. Normal; eats.

Nov. 14. Slight tremors.

Nov. 15. Slight tremors; no salivation. Killed for liver perfusion.

VI. FEMALE, LESS THAN ONE YEAR OLD.

Nov. 12. Glands removed; good recovery.

Nov. 13. Normal.

Nov. 14. Normal.

Nov. 15. Slight tremors. Killed for liver perfusion.

VII AND VIII. MALES, LESS THAN ONE YEAR OLD.

Nov. 12. Glands removed; good recovery.

Nov. 13–22. Foxes normal in every respect; under observation every day, but no symptoms noted. Killed for liver perfusion. Post mortem; a nodule of accessory thyroid found in the chest in VII. No trace of parathyroids.

IX. MALE, LESS THAN ONE YEAR OLD.

Nov. 28. Glands removed; good recovery.

Nov. 29. Normal.

Nov. 30. Normal.

Dec. 1. Slight tremors. Killed for liver perfusion.

X. FEMALE, LESS THAN ONE YEAR OLD.

Nov. 28. Glands removed; good recovery.

Nov. 29. Normal; does not eat.

Nov. 30. Normal; eats.

Dec. 1–3. Normal.

Dec. 4. Slight tremors. Killed for liver perfusion.

XI AND XII. FEMALES, LESS THAN ONE YEAR OLD.

Nov. 28. Glands removed.

Nov. 29–Dec. 20. Foxes apparently normal in every respect.

Dec. 21. Fox XII normal; fox XI shows depression and tremors; does not eat.

Dec. 22. Continued weakness and tremors of fox XI; does not eat.
Dec. 23. Fox XI found dead. Post mortem showed congestion of lungs, possibly pneumonia. No accessory thyroid or parathyroids found.
Dec. 24. Fox XII normal.
Dec. 25. Fox XII normal.
Dec. 26. Found dead, cause unknown. No accessory thyroids found.

The following points may be restated for emphasis:

1. None of the animals exhibited any symptoms within less than thirty-six hours after operation, and only three of them (Nos. III, IV, and V, within forty-eight hours after.

2. The tendency to periodicity in the symptoms, particularly striking in fox III.

3. The apparent absence of all symptoms in foxes II, VII, and VIII, and at least a long delay (three weeks) in development of symptoms in foxes XI and XII.

4. The complete recovery after development of extreme symptoms in fox 1. We have never observed such violent and prolonged convulsions either in dogs or cats, and we fully expected the fox to die any minute.

5. Some foxes exhibit no effects, at least for long periods, and some recover completely after thyroid-parathyroidectomy, no discoverable remnants or accessory glands being left behind. But, of course, we did not make serial sections of the whole neck and chest region of these foxes, so the possibility remains that isolated thyroid or parathyroid cells may have been present in the various fascia, in the lymph gland or the thymus, but we doubt if such cells could have been identified with certainty even if such serial sections had been made.

We can suggest no other explanation for the difference between the results of Vincent and Jolly and ours than that of individual variations in the resistance to the untoward conditions imposed by the removal of the glands. Most of our animals were born and raised in captivity. They were none the less strictly carnivorous. As a matter of fact these foxes will starve for days before they even eat cooked meat.

IV. THE IODINE CONTENTS OF DOG'S THYROIDS.

In connection with the iodine determinations in goitre lymph and on some of the goitrous thyroids used in the experiments above described, we made similar tests on the thyroids of one hundred and two dogs taken at random as brought to the laboratory. It has already been stated that over 50 per cent of these dogs have thyroid enlargements discernible on direct inspection or by palpation, and that apart from the thyroid enlargement these dogs give no apparent evidence of any disease usually involving the thyroids in man — exophthalmic goitre, myxedema, cretinism. To quote the conclusions of Marine and Lenhart, "most of these dogs are normal." Because of this fact and in view of the unsettled question of the relation of the thyroid secretions to iodine, we deemed such a preliminary survey desirable, despite the abundant literature on the subject. Marine and Williams have recently made a similar survey on one hundred and thirteen dogs in Cleveland. Our results are given in Table I.

Before we comment on these results we may state the findings in two cases of carcinoma of the thyroids.[18]

(A) Primary carcinoma of both lobes with metastases in adjoining muscles, and lymph glands, and in the lungs. Weight of fresh gland about 450 gm.

I. per gram of dry gland: 0.335 mgm., total about 34.00 mgm.
I. per gram of dry lymph gland: 0.385 mgm., total about 3.85 mgm.
I. per gram of lung metastases: 0.350 mgm., total about 35.00 mgm.
Total iodine about 72.0 mgm.

(B) Same as case A with the difference of greater extent of the lung metastases. Weight of fresh gland about 400 gm. The iodine test on the thyroid, the lymph glands, and the lung metastases were all negative.

Histological examinations were made by Mr. A. B. Luckhardt showing practically identical pictures in the two cases. The lung metastases showed typical thyroid acini with colloid in both cases, and yet iodine was fairly abundant in one, and absent in the other.

To the above data may be added the results of our tests on the thyroids of eleven normal foxes, less than one year old. The weight of the dry gland (both lobes) varied from 0.3 to 0.7 gm. Total iodine in mgm. (1) 0.184; (2) 0.08; (3) 0.277; (4) 0.123; (5) 0.077; (6) negative; (7) trace; (8) 0.078; (9) 0.046; (10) trace; (11) 0.08.

The most striking thing in the data on dogs is the great individual variations, and the number of thyroids in which iodine is either absent or present in too small quantities to be detected by our method. Our results correspond in general to those of Marine and Williams on the dogs of Cleveland, with the exception that they were able to detect iodine in the thyroids of all their adult specimens. All observers agree that the iodine percentage of the thyroids is subject to great variations, it being absent or present only in traces in the new-born, and generally increasing in quantity until maturity. Marine and Lenhart have recently endeavored to correlate histological structure with the iodine percentage in the thyroid of a number of mammalian genera. They conclude, with Oswald, that the amount of iodine is roughly proportional to the amount of colloid in the gland. Active hyperplasia is therefore poor in iodine, colloid goitre contains more, while normal glands contain the highest iodine percentage per dry weight. This is an important generalization. But the fact seems to us of even greater significance that in the dog these different states of histological structure and iodine content are compatible with apparently normal physiological conditions of the rest of the organs. In other words, in comparing dog and man, the apparent similarity of the colloid and the hyperplasia stages of the thyroids must be put side by side with the equally striking dissimilarity of the "clinical picture" exhibited by dog and man in these conditions. It is well known that myxedema may supervene on exophthalmic goitre in man; active thyroid hyperplasia in dogs not only produces no typical symptoms of exophthalmic goitre, but is not known, so far as we are aware, to terminate in myxedema.

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[18] The diagnosis was made by our colleague Dr. H. G. Wells.

V. THE NATURE OF THE GOITRES IN DOGS IN THE REGION OF THE GREAT LAKES.

According to Marine and Williams thyroid hyperplasia is present in 90 per cent of the dogs of Cleveland, and over 80 per cent of these goitres presents the histological picture of active hyperplasia. The goitre is equally prevalent in males and females. This we can confirm for the dogs of the Chicago region. Dr. Werelius in this laboratory has made an extended histological survey of these goitres, finding about the same percentage of active hyperplasia as that recorded by Marine and Williams. We can also confirm the statement of Marine and Lenhart that these dogs are to all appearances normal, save the goitre.

TABLE I.

Iodine Content of one hundred and two common Street Dogs picked at Winter, Spring, and Summer of 1909. Tests made

No. of dog.	Weight of dog, kg.	Weight of fresh glands, gm.	Weight of dried glands.	Iodine, mgm. per gm. dried gland.	Total iodine, mgm.
1	6.1	....	1.51	negative	....
2	6.6	15.0	3.38	0.072	0.244
3	5.2	22.0	5.21	0.169	0.882
4	5.1	6.0	1.7	0.477	0.811
5	7.2	5.0	1.46	0.210	0.293
6	8.5	7.0	1.72	0.154	0.264
7	4.9	1.15	0.32	trace	trace
8	5.8	13.0	2.93	negative	....
9	5.9	2.8	0.84	0.216	0.180
10	8.6	2.75	0.82	1.308	1.094
11	5.2	1.4	0.34	negative	....
12	5.8	6.1	1.39	0.154	0.190
13	19.6	25.6	6.51	0.508	3.307
14	15.0	10.2	2.5	0.369	0.924
15	13.0	....	1.0	0.508	0.508
16	13.0	5.2	1.67	1.801	3.00
17	13.0	7.0	1.01	0.384	0.385
18	11.0	15.35	3.03	0.338	1.127
19	12.0	3.85	1.1	0.076	0.077
20	17.0	37.45	10.17	1.624	16.523
21	18.0	6.35	1.68	0.862	1.448
22	15.0	10.45	2.91	1.678	4.984
23	14.0	2.75	0.69	0.539	0.385
24	22.0	15.2	4.08	0.354	1.445
25	9.0	2.84	0.83	0.955	0.796
26	10.0	35.2	9.45	1.647	15.571
27	16.0	15.75	4.24	1.155	4.987
28	8.0	2.3	0.50	negative	....
29	9.0	2.70	0.60	0.344	0.215
30	7.0	30.8	7.91	0.292	2.021
31	5.0	8.9	1.98	0.077	0.152
32	10.0	16.48	3.83	0.308	1.179
33	10.0	1.55	0.49	0.677	0.338
34	8.0	2.75	0.71	0.110	0.092
35	8.0	9.35	2.16	0.077	0.166
36	10.0	31.5	6.63	negative	....
37	...	3.45	0.66	0.138	0.092
38	12.0	17.1	3.85	0.169	0.633
39	9.0	93.35	23.0	0.169	3.887
40	9.0	65.5	10.59	negative	....
41	17.0	85.9	16.15	negative	....
42	7.0	16.3	3.86	0.138	0.534
42	10.0	6.6	2.08	1.878	3.907
44	6.0	5.1	1.1	0.070	0.077
45	6.0	1.4	0.37	0.523	0.308
46	12.0	3.6	1.35	2.64	3.52
47	10.0	270.0	80.0	0.077	6.16
48	...	7.0	2.4	negative	....
49	12.0	450.0	...	negative	....
50	8.0	3.0	0.8	0.110	0.092
51	6.0	....	1.9	0.020	0.039
52	10.0	....	2.1	0.026	0.054
53	9.0	....	1.6	negative	....

TABLE I.

Random as they were brought to the Laboratory in the Fall of 1908, the according to the Baumann-Oswald Method.

No. of dog.	Weight of dog, kg.	Weight of fresh glands, gm.	Weight of dried glands.	Iodine, mgm. per gm. dried gland.	Total iodine, mgm.
54	...	326.0	...	negative	....
55	...	50.0	...	trace	trace
56	...	330.0	...	trace	trace
57	...	110.0	23.6	0.85	20.10
58	...	75.0	16.0	0.75	12.00
59	...	98.0	24.5	negative	....
60	4.7	10.0	1.95	trace	trace
61	5.2	4.0	0.55	trace	trace
62	5.2	4.0	3.09	negative	....
63	7.7	3.0	0.34	negative	....
64	3.9	3.0	0.29	negative	....
65	10.6	6.33	1.71	0.237	0.405
66	10.3	3.4	0.85	0.169	0.136
67	11.5	16.93	5.33	4.92	26.223
68 [1]	9.0	823.0	115.74	negative	....
69	5.2	4.22	1.32	negative	....
70	6.2	1.98	0.6	negative	....
71	5.4	2.59	0.58	....	0.770
72	7.2	1.75	0.49	trace	trace
73	4.2	4.8	1.59	trace	trace
74	5.6	8.97	2.88	0.887	2.554
75	4.6	17.24	5.1	negative	....
76	5.8	1.42	0.4	0.500	0.200
77	6.8	0.67	0.27	trace	trace
78	7.1	28.35	7.07	negative	....
79	5.0	....	0.94	negative	....
80	4.4	1.25	0.44	negative	....
81	5.8	14.65	4.69	0.167	0.787
82	4.7	2.75	0.87	trace	trace
83	5.4	2.77	0.70	trace	trace
84	5.3	13.54	3.79	1.001	3.793
85	5.1	7.55	1.58	trace	trace
86	6.2	....	0.63	0.323	0.215
87	6.0	....	0.68	negative	....
88	5.4	....	0.26	0.426	0.107
89	8.2	79.98	28.21	trace	trace
90	12.5	8.01	2.59	0.558	1.446
91	12.0	7.93	1.97	trace	trace
92	10.0	10.20	3.28	0.662	2.172
93	10.0	2.22	0.55	negative	....
94	10.1	16.53	3.66	negative	....
95	6.4	5.49	1.25	trace	trace
96	5.8	....	1.26	0.800	1.009
97	5.7	....	0.54	trace	trace
98	6.4	....	1.14	0.134	0.154
99	16.2	....	3.44	0.483	1.661
100	6.2	....	1.65	0.201	0.338
101	5.5	....	6.20	negative	....
102	...	3.0	0.58	1.040	0.612

[1] No. 68 was cystic goitre containing 500 c.c. fluid. The test for iodine on this fluid was negative.

The general appearance of the dogs and the equal prevalence of the hyperplasia in both sexes seem to indicate that the goitres are of the simple or benign type, despite the apparent hyperplasia of the glands themselves. Moreover, exophthalmic goitre in man is not especially prevalent in endemic goitre districts. Some have even claimed that the toxic goitre is more prevalent on the seacoast. Marine, in identifying the active hyperplasia in these dog goitres with exophthalmic goitre in man, has drawn far-reaching physiological conclusions from histological premises and with too little emphasis on the obvious physiological facts.

Little or nothing is known as to the etiology of these dog goitres. The Great Lakes region does not appear to be an endemic goitre district for man especially or, so far as we know, for any mammal besides the dog. We have never seen palpable goitres or even undoubted thyroid enlargements in cats in this city, and we have examined several hundred cats during the past three years. The cats certainly drink the same water, breathe the same air, and, in general, eat the same kind of foods as the dogs. Yet the goitre is present in 90 per cent of the one species and practically absent in the other.

It is well known that partial removal of the thyroid leads in many cases to hypertrophy of the remainder of the gland. This is probably a compensatory hypertrophy. The histological picture of this hypertrophy is very similar to the type of goitre designated as active hyperplasia, and on this basis Marine and Lenhart conclude that all active thyroid hyperplasia is a compensatory hypertrophy. This physiological generalization, again, is based mainly on histological evidence, and appears to run counter to some generally accepted observations:

1. In the first place, if all thyroid hyperplasia (not including tumors) is compensatory hypertrophy pure and simple, partial or complete removal of hyperplasia thyroids in man ought to aggravate the symptoms of tachycardia, emaciation, and nervousness of exophthalmic goitre. In fact; on that hypothesis the surgical treatment of exophthalmic goitre is distinctly contra-indicated and would constitute one of the most solemn tragedies in the history of medicine. That procedure itself ought to have demonstrated the falsity of the theory of Moebius. Now, this is not the case. The literature certainly shows that in human exophthalmic goitre where the malady has not proceeded to too great exhaustion of the patient, partial removal of the thyroid leads in the majority of cases to a more or less rapid, even if only temporary, diminu-

tion of the symptoms, and in no case to undoubted aggravation of the symptoms. The high death rate from operations in very extreme cases is probably not due primarily to the partial removal of the thyroid, but would result from the general anæsthesia and operation on any other organ.

2. In the majority of cases of exophthalmic goitre thyroid administration intensifies some or all of the symptoms, while on the compensatory hypothesis it ought to alleviate the svmptoms.

3. If all thyroid hyperplasia is a compensatory hypertrophy, removal of all thyroid tissue in normal animals ought to produce, primarily at least, the symptoms of exophthalmic goitre. Marine and Lenhart seem to think that such is the case, and they refer to the indications of increased excitability in completely thyroidectomized monkeys in support of their contention. The unsettled problem of the relation between the thyroid and the parathyroid is a disturbing factor in deciding this point, but the verdict of the literature seems to be to the effect that the removal of the thyroids proper produces none of the cardinal symptoms of exophthalmic goitre in experimental animals. This we can affirm on the basis of our own work in the case of pigeons, rats, mice, and rabbits, and in some instances in foxes. It is an open question whether complete thyroidectomy can be made in carnivora (dogs, foxes, and cats) without removal of most of the parathyroids at the same time, but the workers who claim they can and have done so state that no other symptoms are produced than a gradual onset of myxedema, and even these appear to be absent in many cases.[19]

As regards the primary symptoms of thyroidectomy in monkeys, we can state, on the basis of our own work, that tremors and convulsions may occur, but there is no tachycardia or emaciation. And it must be remembered that in these operations the parathyroids are also removed, hence the transient tremors may be due to that cause alone.

The fact of hypertrophy of thyroid remnants in normal animals indicates the need under normal conditions of the thyroids or their products, but there is no evidence that failure to meet this need in the otherwise normal animal leads primarily to the symptoms of exophthalmic goitre.

4. Experimental hyperthyroidism, so far as it goes, seems to support the theory of Moebius. In man as well as in experimental animals

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[19] VINCENT and JOLLY: Journal of physiology, 1906, xxxiv, p. 295; UNDERHILL and HILDITCH: This journal, 1909, xxv, p. 43.

thyroid administration augments the oxidative processes and thus leads primarily to loss of body weight. It appears not to be definitely determined whether it also leads to increased nitrogen elimination.[20] In the human it is said to produce tachycardia and nervousness. Temporary tachycardia is also produced in dogs. It would thus seem that thyroid administration reproduces in a mild form the three main symptoms of exophthalmic goitre. But, so far as we are aware, the condition of extreme exophthalmic goitre in the human has never been produced experimentally by thyroid administration in any animal. Hunt failed to detect thyroid substances in the blood of guinea pigs after excessive thyroid administration, a fact that has been confirmed by Mr. Lussky in our laboratory. The work of von Fürth and Schwarz seems to indicate the development of immunity or tolerance to thyroid substance, administered subcutaneously, in normal dogs. These facts seem to indicate that the etiology of the symptom complex of exophthalmic goitre in man is too complex for any one of the hypotheses so far advanced. Unfortunately, exophthalmic goitre is rare in experimental animals. Nothing is known as regards its occurrence in monkeys in their wild state. But it seems to us that some light ought to be shed on the question by a more extensive study of the effects of excessive thyroid administration in birds and mammals covering longer periods than has so far been carried out.

VI. THE RELATION OF THE THYROID TO THE PARATHYROID AND THE SIGNIFICANCE OF THE THYROID AND THE PARATHYROID COLLOID.

We do not propose to go into any detailed discussion of the literature on the physiological relation of the thyroid and the parathyroid, as this has recently been done by Forsyth,[21] and to some extent also by MacCallum and Voegtlin.[22] The data are contradictory as regards structure, chemistry, and function. It seems to us that the question of the relation between the thyroids and the parathyroids is inseparable from that of the significance of the thyroid colloid, because young thyroids and in most cases the parathyroids appear to function without colloid production.

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[20] SCHÖNDORFF: Archiv für die gesammte Physiologie, 1897, lxvii, p. 395.

[21] FORSYTH: Quarterly journal of medicine, 1908, ii, pp. 150, 287.

[22] MACCALLUM and VOEGTLIN: Journal of experimental medicine, 1908, xi, p. 118.

1. **Structure.** — Kishi,[23] Vincent and Jolly,[24] and Forsyth maintain that we have at present no microchemical criterion which will enable us to differentiate young thyroid tissue, the interfollicular layers of cells in adult thyroids and parathyroid tissue. There seems to be, for the present, no escape from that position. The only distinguishing feature appears to be the follicular arrangement and the colloid and possibly the presence of droplets of fats in parathyroid cells. But that does not apply to the thyroids of the new-born, as in these neither follicular structure nor colloid is present. Follicular structure and colloid are also present in many cases in the parathyroids themselves. Vincent and Jolly, and Forsyth cite many examples. Krause[25] found this condition ordinarily in the posterior pair of parathyroids in the rabbit. Rulison[26] found colloid-filled follicles in two out of seventeen human parathyroids. Forsyth claims that the accessory parathyroids are gradually changed into typical thyroid tissue. Halpenny and Thomson[27] traced the transformation of the parathyroids into typical thyroid tissue following extirpation of the thyroids. This last experiment suggests that the transformation is of a compensatory nature. And, lastly, both follicular structure and colloid are absent in certain physiological states of the adult thyroid. The question of correspondence in the structural changes in the two gland groups in myxedema and goitre is an open one, Forsyth claiming that the parathyroids do, and Marine and Lenhart that they do not, change *pari passu* with the thyroid.[4] Haberfeld and Schilder[28] describe compensatory hypertrophy in the parathyroids of rabbits. To recapitulate: follicular structure and colloid are generally, but not always, present in the thyroids; they are generally, but not always, absent in the parathyroids; parathyroid tissue may be transformed into typical thyroid tissue and *vice versa*, at least as regards structure.

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[23] Kishi: Virchow's Archiv, 1904, clxxvii, p. 260; 1906, xxxiv, p. 295.

[24] Vincent and Jolly: Journal of physiology, 1904, xxxii, p. 65.

[25] Krause: Anatomie des Kaninchens, p. 212.

[26] Rulison: Anatomical record, 1909, iii, p. 399.

[27] Halpenny and Thomson: Anatomische Anzeiger, 1909, xxxiv, p. 376.

[28] Haberfeld and Schilder: Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie, 1909, xx, p. 727.

2. **Chemistry.** — Iodine has been reported both present and absent in the parathyroids. Both series of results are in all probability correct. Since part of the iodine of the thyroid complex seems to be fairly definitely related to the colloid, it is possible that the positive results reported were obtained on parathyroids relatively rich in colloid. Iodine is ordinarily present in the adult thyroid, but it may be absent.[29] It is ordinarily absent in the thyroids of the young or the new-born. It may be present in the gland in the absence of colloid.

The physiologically active principles or secretions of the glands appear to be different. In the case of the parathyroids it appears to be a nucleo-protein (Beebe, MacCallum, and Voegtlin); in the case of the thyroid it is commonly held to be an iodized globulin (Baumann). But neither of these two conclusions is established. The present tests for the thyroid secretions may be primarily tests for iodine; and the tests for the parathyroid secretions are as yet insufficiently controlled.

3. **Function.** — There is no doubt that in many animals the removal of the parathyroid leads to a series of symptoms usually designated as parathyroid tetany, generally ending in death. Depression appears to be an equally important factor in the symptom complex.[30] But in most carnivora one cannot be sure that all the parathyroid tissue is removed without removing at the same time practically all the thyroid tissue. And the fact that isolated groups of thyroid and parathyroid cells may be lodged anywhere between the level of the base of the tongue and that of the diaphragm makes it impossible to affirm that all thyroid tissue has been removed in any one case, as serial section of the neck and whole chest region is practically out of the question even in our smallest adult mammals. Thus, whether temporary or fatal symptoms do or do not follow extirpation of all discoverable parathyroid tissue, in neither case can we affirm that *all* parathyroid tissue had been removed.

Vincent and Jolly obtained typical tetany symptoms in a certain percentage of dogs and cats on removal of the thyroids alone, but in our experience this is very difficult to accomplish in these animals without great injury to or removal of the parathyroids, and most of the recent workers conclude that simple thyroidectomy in dogs and cats results only in the gradual development of cretinoid conditions. Our own results show that at least in pigeons, rats, mice, and rabbits, thyroid extirpation does not produce any of the tetany symptoms, but these symptoms may be absent in rodents even after extirpation of all detectible parathyroid tissue.[31]

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[29] Hunt and Seidell: Hygienic Laboratory, Bulletin No. 47, 1909, pp. 32–45.

[30] Carlson and Jacobson: This journal, 1910, xxv, p. 403.

The grafting experiments of Halsted[32] seem to indicate a degree of functional independence of the parathyroids, the graft not taking unless a specific parathyroid insufficiency had been produced, but the work of Thomson, Leighton, and Swartz[33] points even more strongly to vicarious function or interchange of function, as the transplantation of a portion of the thyroid into the red bone marrow in dogs prevents the tetany and allied symptoms on extirpation of all the parathyroids.

Myxedema and cretinism have never been produced by parathyroid insufficiency, but that may be due to the fact that death supervenes too rapidly for these conditions to develop. ✓

Both thyroid and parathyroid have this in common, that they become of gradually less importance or rather less essential to the organism with age. But one of the most troublesome factors is the *variable* effects of hypothyroidism in adults in the different groups of vertebrates, as well as in individuals of the same group. Myxedema in man is generally considered to be due to thyroid insufficiency, but these symptoms do not always follow extirpation of the glands in otherwise normal birds and mammals. The conclusions of Vincent and Jolly on this point may be criticised on the score that they did not keep their animals for a sufficient length of time after the operations. But our own results show that myxedema does not develop in thyroidectomized rabbits at least in seven months. We have at present in our laboratory a rhesus monkey on which the operation of (presumably) complete thyroidectomy was performed October 12, 1909, but there is to date no evidence of even incipient myxedema. While thyroid insufficiency in the young appears to cause arrest of growth and cretinoid conditions in all species studied, in adults hypo- and hyperthyroidism appear to have most pronounced effects in the human species.

Two facts seem to be indicated by the above brief review, namely: 1. The structure of the thyroids and the parathyroids is primarily the same, and even in the adult state the one may become transformed into the other; 2. There is probably both quantitative and qualitative differences in their function. This relation between the thyroid and the parathyroid thus appears to be similar to that between the Islands of Langerhans and the ordinary parenchyma in the pancreas, as revealed by the recent work of Dale,[34] and of Vincent and Thomson.[35] The fact that the one type of gland can be transformed into the other does not argue against qualitative difference in their function. But are we forced to assume such a difference, even in part, in the case of the thyroid and the parathyroid? It seems so, at present. It does not seem possible to account for all the facts on the basis of difference of degree alone, however tempting such an hypothesis may be. Thus the older view that the parathyroids are merely young or embryonic thyroids might be coupled with the fact that the importance of the thyroid to the organism diminishes with age or degree of differentiation of the gland. The thyroid cells might thus be conceived of as undergoing progressive differentiation from embryonic or parathyroid condition to the state of the interfollicular cells of the thyroid, and these again into the follicular cells whose life is about to end in colloid degeneration. This might account for the rapid and severe effects of the removal of the young gland (parathyroid) as compared with the much less severe and more gradually developing symptoms following removal of the older gland (thyroid). But the two series of effects are so different as to suggest more than a difference in degree of gland insufficiency. It is indeed probable that even under normal conditions the cells of the parathyroids do a portion of the work of the thyroids and *vice versa*, but this point is not capable of proof by our present methods because of the small fraction of the normal glands that suffices for the maintenance of the normal physiological equilibrium.

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[31] Haberfeld and Schilder: Mitteilungen aus den Grenzgebieten der Medizin und Chirugie, 1909, xx, p. 727.

[32] Halsted: Journal of experimental medicine, 1909, xi, p. 175.

[33] Thomson, Leighton, and Swartz: Journal of medical research, 1909, xxi, p. 135.

4. **The significance of the colloid.** — We have seen that the colloid is generally absent in the parathyroids, in young thyroids, and in certain conditions of thyroid hyperplasia. This seems to show that follicular structure and colloid is not a *sine qua non* for thyroid-parathyroid functioning. But the question still remains as to the physiology of the colloid when present. The colloid has probably a double origin, namely,

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[34] DALE: Philosophical transactions of the Royal Society, 1904, cxcvii, p. 25.

[35] VINCENT and THOMSON: Internationale Monatsschrift für Anatomie and Physiologie, 1907, xxiv, p. 61.

(1) actually secreted by the follicular cells, (2) produced by colloidal degeneration of the follicular cells. The latter process is similar to the mechanism of secretion in the sebaceous glands. It has already been pointed out that the view of Langendorff and Hürthle as to the presence of canals between the follicular cells serving as outlets for the colloid is untenable. In order to reach the lymph and the blood the colloid must therefore pass back through the same cells that secreted it originally. That this actually occurs is shown by the glands in active hyperplasia free from the colloid, as it is known that this condition may supervene on both normal and colloid glands. We do not know what changes the colloid undergoes in the process of absorption and reverse elimination by the follicular cells.

So far as we are aware, the thyroid colloid is the only instance in the whole animal kingdom where a supposed physiological secretion is first eliminated from the cells in one direction, then absorbed by the same cells and eliminated in the reverse direction. This process resembles that of formation, resolution, and absorption of pathological exudates and transudates, or the removal of intracellular by-products and waste deposits. It is true that in the intestines water and some solids in solution probably pass in either direction through the same epithelial cells, but these substances are not specific products of the cells. Colloid is formed in the pars intermedia of the hypophysis,[36] but this colloid does not double back on its route of elimination like the thyroid colloid.

Even if we assume that the colloid constitutes or contains the essential gland secretions, the fact that it has to be reabsorbed and pass in the reverse direction through the living cells leaves it an open question in what form these secretions ultimately reach the blood. On this view the colloid would be in reality storage material destined to be elaborated to physiologically important secretions by the gland cells, analogous to secretion granules or proferments. If this is a correct interpretation, it is, again, the only instance in the animal kingdom where such material is stored up *intercellularly*. Iron, glycogen, and fat, substances stored extensively in different organs, are all stored *intracellularly*.

The fact that the iodine storage capacity of the thyroid appears to be in general proportional to the colloid content and that active hyperplasia in dogs can be quickly transformed into colloid goitre by the administration of iodine (Marine and Lenhart) suggests the detoxication hypothesis of von Cyon. Iodine in all forms when in sufficient quantity is a poison to the organism. And the mechanism of immediate protection appears to be its fixation in the tissues rather than its elimination in the urine. Now, the fact is that all other tissues appear to fix the iodine without the aid of any colloid. But this is no serious objection to the detoxication hypothesis, since the same can be done by the thyroid. And it is significant that normal and colloid thyroids are apparently capable of fixing more iodine per quantity of tissue than any other organ in the body. The iodine fixation by other organs does not appear to have any significance in relation to the specific functions of the organs. This apparent special device for iodine detoxication or iodine storage in the thyroid may suggest such a relation, but it does not prove it. The hypothesis would explain the appearance of active hyperplasia on iodine starvation, but it runs counter to the equally important fact of the abundance of colloid without a trace of iodine. Moreover, the microchemically identical colloid of the pars intermedia of the hypophysis appears to have no specific iodine fixation properties.[37] Our own tests for iodine on ox hypophysis obtained from the Chicago stock yards have all given negative results.

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[36] Herring: Quarterly journal of experimental physiology, 1908, i, p. 187.

When one examines the enormously enlarged and irregularly shaped colloid follicles of certain types of goitre, one can hardly refrain from classifying them with pathological processes and cyst formations; but this hypothesis seems untenable when the field is shifted to the normal thyroid with its regular follicles. It seems far fetched to look upon them as incipient cysts.

The thyroid colloid thus presents one of the most unique and baffling problems in the organ physiology of animals. No aid in its interpretation has so far been obtained from the colloid of the hypophysis because the experiments of Schäfer and Herring[38] are not conclusive, in view of Halliburton's[37] and of Cushing's results.[39] No hypothesis advanced to account for its function seems consistent with all the facts so far known. But this much seems clear — too great emphasis has been placed on the supposed essential relation of the colloid to the special

[37] Simpson and Hunter: Proceedings of the Society for Experimental Biology and Medicine, 1909, vii, p. 11; Halliburton, Chandler and Sikes: Quarterly journal of experimental physiology, 1909, ii, p. 289.

[38] Schäfer and Herring: Philosophical transactions, 1906, cxcix, B, p. 1.

[39] Reford and Cushing: Johns Hopkins Hospital Bulletin, 1909, xx, p. 105.

secretions of the glands. The glands appear most essential in the young when they contain little or no colloid; they function normally as well as in condition of active hyperplasia with little or no colloid, and there is little evidence that the great mass of interfollicular cells take any part in the colloid production.

VII. SUMMARY.

Lymph production in the thyroids.

1. The lymph production in the normal thyroid is relatively slight. It is less than in salivary glands of corresponding size. In average-sized dogs with normal thyroids it is estimated that less than 2 c.c. of thyroid lymph is emptied into the blood in twenty-four hours.

2. All types of thyroid enlargement (including tumors) are accompanied by increased output of lymph from the thyroids. The increased lymph output is roughly proportional to the size of the gland, and seems therefore to be correlated with growth and blood supply. There is no relation between iodine content of the glands and the lymph production. Whether there is any correlation between the lymph production and the specific gland activities remains an open question. In goitres of the size of a kidney or the spleen the lymph production is much greater than in the latter organs. In large goitres the quantity of the thyroid lymph poured into the blood in twenty-four hours is greater than that secreted in normal thyroids in the course of a whole week.

3. So far as examined the general character of the goitre lymph appears to be the same as that of the common neck lymph, except that it contains fewer lymphocytes. In cystic goitres it may contain traces of the cystic fluid.

Thyroid secretions in thyroid lymph.

4. Chemical tests for iodine in thyroid lymph were negative. Iodine compounds are either absent or present in too minute traces for chemical detection. Evidence is cited showing that the relation of iodine to the thyroid secretions is yet an open question.

5. Intravenous injections of thyroid lymph in dogs under anæsthesia have no specific effects on the circulation. Evidence is cited that thyroid extracts so far prepared have no specific action on the circulation.

6. Intravenous injections of thyroid lymph in dogs not under anæs-

thesia causes a temporary rise in the temperature, and in most cases some augmentation and irregularity of the pulse. The same effects are produced by the injection of blood from the same dogs. These effects are therefore probably non-specific and cannot be identified with symptoms of hyperthyroidism.

7. Complete elimination of the thyroid and parathyroid lymph for thirty-six to forty-eight hours in normal foxes does not induce the symptoms of thyroid-parathyroidectomy.

8. The aceto-nitrile test of Hunt on thyroid lymph gave uniform negative results. The results of Hunt on feeding mice with thyroid preparations are confirmed. But the mice fed on thyroid lymph showed no increased immunity to the aceto-nitrile. Evidence is cited showing that the aceto-nitrile test is probably a test for iodine compound in general rather than a test for specific thyroid secretions.

9. Complete thyroidectomy in foxes leads to: (1) tetany, cachexia, and death; (2) tetany and depression followed by complete recovery as long as observed (two to four months); (3) no symptoms either of tetany or myxedema for at least one to four months. The significance of the individual and generic variations in the resistance to thyroid-parathyroid deficiency is discussed.

The nature of the dog goitres in the Great Lakes region in the United States.

10. The iodine content of the thyroids is extremely variable. Iodine may or may not be present in thyroid carcinoma and in the lung and lymph gland metastases. Iodine may be absent, or, more accurately, not present, in detectable quantities in the thyroids of dogs which to all appearance are normal animals.

11. The percentage of active hyperplasia in dog goitre in the Chicago region is about the same (Werelius) as that recorded for the Cleveland region (Marine). The Lakes region appears to be an endemic goitre district mainly for dogs. It certainly is not a goitre region for cats.

12. These dogs with active thyroid hyperplasia according to histological criteria exhibit none of the symptoms of exophthalmic goitre in man. There is no relation of these goitres to sex. The dogs are to all appearance normal, save the goitre. These dog goitres thus seem to resemble simple or benign goitres in man, despite the structure of the thyroids themselves.

13. Evidence is cited rendering Marine's hypothesis of compensatory hypertrophy as the essential factor in all active hyperplasia of the thyroids insufficient and untenable.

The significance of the thyroid and parathyroid colloid.

14. Evidence is cited showing (1) that the thyroid may assume parathyroid structure and function and *vice versa*, and (2) that there is in all probability both a quantitative and qualitative difference in function of the thyroid and the parathyroid under normal conditions.

15. Evidence is cited showing that both thyroid and parathyroid perform their functions with or without colloid or iodine in the glands; and that the present hypotheses concerning the relation of the colloid to the specific thyroid and parathyroid activities have little or no basis in fact.

The path of absorption of the thyroid-parathyroid secretion.

16. We have as yet no undoubted or adequate test for the thyroid-parathyroid secretions in the body fluids. When such tests are discovered, it will in all probability be found, in view of what is now known concerning the distribution and paths of absorption of all other internal secretions, that these secretions are more concentrated in the blood than in the lymphs, and that they enter the blood directly rather than indirectly through the gland lymphatics.

CONTRIBUTIONS TO THE PHYSIOLOGY OF LYMPH. — XII. METHODS OF INDUCING THE APPEARANCE OF POLYMORPHONUCLEAR LEUCOCYTES IN THE LYMPH.

By J. R. GREEN.

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I. INTRODUCTION.

THE work here reported was undertaken at the suggestion of Professor Carlson with the view of devising some method for determining the relation of the polymorphonuclear leucocytes to enzymes and antibodies in the blood and lymph.

Normal lymph does not contain polymorphonuclear cells.[1] Will there be any qualitative or quantitative change in the lymph with the introduction of polymorphonuclear leucocytes under conditions not involving injury to, or a changed physiological state in, the blood and lymph capillaries? Is it possible to induce the appearance of polymorphonuclear leucocytes in the lymph without such changed conditions in the capillaries?

If this is not the case, changes in the lymph *pari passu* with the appearance of polymorphonuclear leucocytes in it may be due to the changed condition of the blood and lymph capillaries and consequent establishment of a new equilibrium level between the lymph and the blood, rather than the presence of the polymorphonuclear cells.

II. LITERATURE.

The literature is referred to in greater detail in a recent paper by Davis and Carlson.[2] The work of MacCallum, Sabin, and others renders it highly probable that the lymph circulates in a closed system of capillaries and vessels quite independent of the blood vessels and tissue spaces. Weidenrich, Leaf, Rous,[4] and others maintain that there is a capillary anastomosis between the thoracic duct and the veins in the superior mediastinum, in many cases. It is difficult to accept this as normal in view of the fact that erythrocytes are not present in the normal lymph from the thoracic duct.

Likewise it has been shown that the cellular elements of the blood and lymph are probably distinct in origin.[1] According to Muir,[3] the eosinophile and neutrophile granular cells pass directly into the blood stream and not into the lymph. Biedl and Deastello conclude that both eosinophile and neutrophile leucocytes, if present in the lymph, must come directly from the blood. Winternitz[5] induced polymorphonuclear leucocytes into the lymph of dogs by the injection of large quantities of turpentine into the paw of the animal. The interesting observations of Clark[6] on the living lymphatics of the frog larva indicate that the passage of blood cells from the tissue spaces into the lymph capillaries may be brought about by amœboid movements of lymph capillary wall.

III. METHODS.

1. **Collection of lymph.** — The same procedures were used as described by Davis and Carlson.[2]

2. **Smears and staining.** — The smears were dried and fixed by placing them in absolute alcohol for an hour or more. The film was then stained with Giemsa's stain and examined with the oil immersion lens without mounting.

3. **Methods of inducing polymorphonuclear leucocytes in the lymph.** — (*a*) *Infection on one side of the head or neck.* Staphylococcus aureus was injected into the parotid gland and the side of the face. The neck lymph was collected after twenty-four or forty-eight hours and its cellular elements compared with that from the normal side.

(*b*) *Passive hyperemia.* The external jugular vein was ligated on one side under aseptic conditions and lymph collected from the neck lymphatics after twenty-four hours.

(*c*) *Edema from skin irritants.* Oil of mustard was applied to one side of the face and jaw, and after twenty-four hours the lymph collected from both neck lymphatics as before.

(*d*) *Massage.* Cannulas were inserted in the neck lymphatic trunks and a sample of lymph taken before massage and again after massage of head and neck for fifteen to sixty minutes.

(*e*) *Irritants in the stomach.* A heavy suspension of ground mustard seed in water was introduced by the stomach tube and after six hours lymph collected from the thoracic duct.

IV. RESULTS.

1. Examination of the neck lymph of five normal dogs showed absence of polymorphonuclear leucocytes — confirming the work of Davis and Carlson, and similar observations by Muir.

2. Staphylococcus infection. (*a*) Three experiments with parotid gland infection showed many polymorphonuclear leucocytes in the lymph of the infected side and none on the opposite side. A few erythrocytes were present in two of the experiments.

(*b*) Two experiments with superficial infection on one ear of four days' standing. Polymorphonuclear leucocytes and transitional forms were found, but no erythrocytes.

3. Passive hyperemia by ligation of the external jugular vein on one side twenty-four hours before. Four experiments gave a few — up to forty per cent polymorphonuclear leucocytes. In the one with forty per cent there was a slight blood admixture.

4. *Edema from skin irritation.* — Four experiments gave four to twelve per cent polymorphonuclear leucocytes on the edematous side. There was no blood admixture.

5. *Massage.* — Five experiments. If polymorphonuclear leucocytes are already present in the lymph, massage increases their number temporarily, just as in the case of the lymphocytes. But when they are not present, thirty to sixty minutes' vigorous massage fails to cause their appearance. In fact, they do not appear in the lymph until the massage is so vigorous and long continued as to cause admixture of blood, evidently from rupture of or injury to the capillaries.

6. *Irritants in the stomach.* — Five experiments. A water suspension of mustard in the stomach seems to favor the admixture of blood in the thoracic lymph. There was an abundance of polymorphonuclear leucocytes in all of the experiments, but in only one case was the lymph entirely free from erythrocytes.

V. DISCUSSION OF THE RESULTS.

1. The methods that yielded positive results produced, in all probability, an altered physiological condition of the walls of the blood and lymph capillaries. This altered state of the separating membranes may suffice to account for the passage of the polymorphonuclear cells and of tissue lymph of such a character as to accelerate the infraction or the chemotaxis.

2. Since all the devices which have so far proved efficient in causing the appearance of polymorphonuclears in the lymph involve changed physiological conditions in the membranes that separate the blood and lymph, there appears to be little hope of throwing light on the relation of the polymorphonuclear leucocytes to enzymes and immune bodies in the body fluids by the introduction of these cells into the fluids in which they are normally absent.

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[1] CLELAND: Transactions of the Pathological Society of London, 1905, lvi, p. 381.

[2] DAVIS and CARLSON: This journal, 1909, xxv, p. 173.

[3] MUIR: Transactions of the Pathological Society of London, 1902, liii, p. 379.

[4] ROUS: Journal of experimental medicine, 1908, x, p. 238.

[5] WINTERNITZ: Archiv für experimentelle Pathologie und Pharmakologie, 1905, xxxvii, p. 213.

[6] CLARK: Anatomical record, 1909, iii, p. 183.

THE EFFECT OF THYROIDECTOMY ON THE DEVELOPMENT OF ACTIVE IMMUNITY IN RABBITS.

By C. A. FJELDSTAD.

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IT is generally held that thyroidectomized animals and cretinoid patients exhibit a diminished resistance to infections. It would seem that this might be due to either or both of two conditions: (1) there may be a depression of the immunity reaction; (2) there may be changes in the external and internal epithelial surfaces of the body of such a character as to facilitate the entrance of bacteria. These experiments deal with the first condition.

The investigation of this problem was begun by Dr. Carlson and Dr. Hektoen on dogs, but dogs did not prove favorable material, because of the practical impossibility of removing the thyroids completely without removing the parathyroids at the same time, with subsequent development of tetany and death in five to ten days. This difficulty is not encountered in the rodents; and at Dr. Carlson's suggestion I took up the problem using the rabbit instead of the dog.

LITERATURE.

The literature seems to show that thyroidectomy diminishes at least some of the oxidative processes in the body; and that, conversely, thyroid administration augments, temporarily at any rate, the oxidative processes. In view of these facts it is obvious that active immunity in thyroidectomized animals might throw some light on the relation of the oxidative processes to the production of immune bodies.

Fassin,[1] working with thyroidectomized dogs and rabbits, finds a "marked diminution in the hemolytic as well as the bacterial alexine, never complete disappearance." *Thyroid* administration was found to increase the concentration of complement in the serum. This author also found that death of the rabbits followed thyroidectomy "ordinarily rather rapidly," a result not in accord with those of other observers (including those of ours) on the effects of thyroidectomy in rodents. Marbé[2] studied the normal opsonins in thyroidectomized animals as well as in animals to which a certain amount of sheep's thyroid was fed. The general conclusion reached is that the "opsonic index of serum undergoes important changes, notably a diminution following thyroidectomy, the opsonic index rising, on the contrary, in the animal subjected to experimental thyroid opotherapy." These experiments concern the normal opsonins and complements, not the opsonins and complement in active immunity. The results obtained by these authors have not been verified as yet.

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[1] FASSIN: Comptes rendus de la Société de Biologie, 1907, lxii, pp. 388, 467, 647.

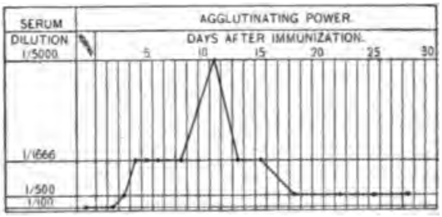

Figure 1. — Rabbit 1. Immunization 7 days after thyroidectomy.

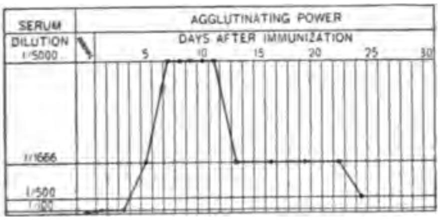

Figure 2. Rabbit 2. — Immunized 22 days after thyroidectomy.

Methods.

Eighteen rabbits were used in this series of tests. Thyroidectomy was performed under ether anæsthesia in twelve, while the other six were "controls." On the second, fourth, seventh, twelfth, twenty-second, and fortieth days after thyroidectomy, animals were immunized against the typhoid bacillus. Serum from each rabbit was collected at two to three day intervals for twenty to thirty days, each complete series being tested at one time, as recommended by Dr. Hektoen.

Results.

The results of four typical series may be cited by tabulation (see Tables I to IV).

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[2] Marbé: Comptes rendus de la Société de Biologie, 1908, lxiv, pp. 1058, 1113; 1909, lxv, p. 612; 1909, lxvi, p. 1073.

TABLE I.

Rabbit 11. Immunization seven Days after Thyroidectomy.[1]

Serum dilution.	Agglutinating power.													
	Normal.	Days after immunization.												
....	..	2	3	4	5	6	8	11	13	15	18	22	25	28
1/5000	0	0	0	0	0	0	0	+	0	0	0	0	0	0
1/1666	0	0	0	+	+	+	+	+	+	+	0	0	0	0
1/500	0	0	+ ?	+	+	+	+	+	+	+	+	+	+	+
1/100	+	+ ?	+	+	+	+	+	+	+	+	+	+	+	+
1/50	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1/10	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[1] +, distinct agglutination; 0, absence of agglutination; + ?, doubtful agglutination.

TABLE II.

Rabbit 15. Immunized twenty-two Days after Thyroidectomy.[1]

Serum dilution.	Agglutinating power.													
	Normal.	Days after immunization.												
....	..	1	3	5	7	8	9	10	11	13	16	19	22	24
1/5000	0	0	0	0	+	+	+	+	+	0	0	0	0	0
1/1666	0	0	0	+	+	+	+	+	+	+	+	+	+ ?	0
1/500	0	0	0	+	+	+	+	+	+	+	+	+	+	+
1/100	0	+	+	+	+	+	+	+	+	+	+	+	+	+
1/50	0	+	+	+	+	+	+	+	+	+	+	+	+	+
1/10	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[1] +, distinct agglutination; 0, absence of agglutination; + ?, doubtful agglutination.

TABLE III.

Rabbit 11. Re-immunized Forty Days after Thyroidectomy.[1]

Serum dilution.	Agglutinating power.											
	Normal.	Days after immunization.										
....	..	1	3	5	7	8	9	10	11	12	14	18
1/5000	0	0	0	0	+ ?	+	+	+ ?	0	0	0	0
1/1666	0	0	0	+	+	+	+	+	+	+	+	+
1/500	0	0	0	+	+	+	+	+	+	+	+	+
1/100	+	+	+	+	+	+	+	+	+	+	+	+
1/50	+	+	+	+	+	+	+	+	+	+	+	+
1/10	+	+	+	+	+	+	+	+	+	+	+	+

[1] +, distinct agglutination; 0, absence of aggutination; + ?, doubtful agglutination.

TABLE IV.

Rabbit 15. Control. Immunization without Thyroidectomy.[1]

Serum. dilution.	Agglutinating power.											
	Normal.	Days after immunization.										
....	..	1	3	5	7	9	12	15	18	21	25	30
1/5000	0	0	0	0	+	0	0	0	0	0	0	0
1/1666	0	0	0	+ ?	+	0	0	0	0	0	0	0
1/500	0	0	0	+	+	+	+	+	0	0	0	0
1/100	0	0	+ ?	+	+	+	+	+	+	+	+	+
1/50	0	+ ?	+	+	+	+	+	+	+	+	+	+
1/10	+	+	+	+	+	+	+	+	+	+	+	+

[1] +, distinct agglutination; 0, absence of agglutination; + ?, doubtful agglutination.

The results are typical of the whole series. They show that there is no detectable difference in the degree of immunity, in the rapidity of its development, nor in its duration in normal and thyroidectomized rabbits. If the internal secretion of the thyroids has a marked influence on the formation of antibodies, there should be a decrease in the immunity reaction *pari passu* with the disappearance of this secretion from the blood.. But it must be remembered that the rate of disappearance of this secretion from the blood and organs after thyroidectomy is not yet known. Rabbit 1 was immunized seven days after thyroidectomy, rabbit 2 after twenty-two days had elapsed, and rabbit 11 re-immunized forty days after operation, yet the *immunity curves* are *all similar*, *all apparently normal.*

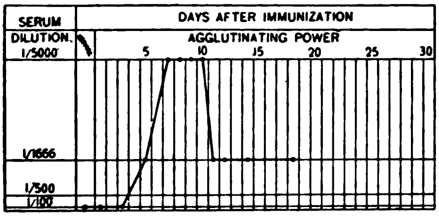


Figure 3.—Rabbit 1. Re-immunized 40 days after thyroidectomy.

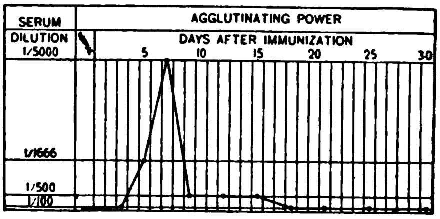


Figure 4.—Rabbit 1. Control. Immunization without thyroidectomy.

Conclusion.

Removal of the thyroids from the rabbit does not, at least during the first month, appreciably affect the formation of immune bodies (more specifically the agglutinins). It is therefore probable that any increased susceptibility to infection in such animals must be ascribed to causes other than the depression of the immunity reaction.

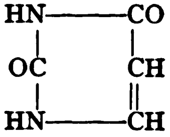
Special thanks are due Dr. Carlson for valuable suggestions.

THE METABOLISM OF SOME PYRIMIDINE DERIVATIVES.

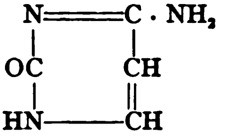
By LAFAYETTE B. MENDEL and VICTOR C. MYERS.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

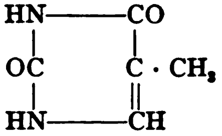
THE demonstration of the wide-spread occurrence of pyrimidine derivatives as constituents of typical nucleic acids has awakened an interest in the physiological action of the pyrimidines and in their behavior in metabolism. Of the three compounds, uracil, thymine, and cytosine, the last two have been isolated more frequently than has uracil. The relation of these three derivatives is shown by the formulas below:



uracil



cytosine



thymine

Some writers have considered uracil to be a secondary product, derived from cytosine or thymine, and not existing preformed in the nucleic acid complexes.[1] This is analogous, in the relation of uracil to cytosine at least, to the more generally accepted view regarding the occurrence of aminopurines as primary constituents of true nucleic acids, the finding of hypoxanthine and xanthine being ascribed to secondary reactions. That such changes can be brought about as the result of autolytic changes, presumably owing to the activity of enzymes, was suggested by experiments of Jones[2] and Levene.[3] For example, after autodigestion of spleen and thymus, the nucleic acid of which yields thymine and cytosine by hydrolysis with acids, uracil was found by Jones, while cytosin was no longer detectable. Likewise Levene could find no cytosine after autolysis of spleen and pancreas, while the content of uracil was decidedly increased. It should be emphasized, on the other hand, that uracil has been isolated from the acid hydrolysis products of two nucleic acids of vegetable origin — from the yeast, by Ascoli,[4] and from the wheat embryo (triticonucleic acid), by Osborne and Harris.[5] Mandel and Levene[6] have similarly isolated uracil in place of thymine from the nucleic acid obtained from fish eggs. Any final generalization as to the primary pyrimidines of nucleic acids is therefore premature at the present time.

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[1] Cf. Schittenhelm and Brahm: Oppenheimer's Handbuch der Biochemie, 1909, i, p. 611.

[2] Jones: Zeitschrift für physiologische Chemie, 1904, xlii, p. 35.

[3] Levene: This journal, 1904, xi, p. 437; Zeitschrift für physiologische Chemie, 1904, xli, p. 397.

Burian[7] has raised the question whether the nucleic acids actually contain a preformed cytosine group or whether the latter can be formed from purines by the action of strong sulphuric acid in the presence of carbohydrates. This opens a debate regarding the possible origin of pyrimidines from purines in the usual experimental manipulations. Burian's experimental evidence has been attacked by various investigators;[8] but conclusive experiments indicating that in the hydrolysis of nucleic acids the pyrimidines have a primary origin, have been published by Osborne and Heyl.[9] They rightly remark that the real problem is not, *can* the pyrimidines be obtained from purines, but, *are* they present as such in the molecule of nucleic acid? Their evidence leaves little doubt as to the reliability of the current view.

Although the numerous investigations on the metabolism of the nucleic acid complexes in the animal organism have served to elucidate the fate of some of the constituent groups, notably the purines and the phosphoric acid derivatives, almost nothing is known regarding the rôle of the pyrimidines. It seems evident that they are liberated by enzymes from the organic union in which they exist in the nucleo-proteins. Several questions at once suggest themselves. Are the "physiological" pyrimidines transformed by tissue enzymes in ways analogous to those experienced by the purines? Can these pyrimidines be synthesized to purines in the animal organism, *i. e.*, can they function as purine precursors? Are pyrimidines originating in endogenous or exogenous nucleic acid metabolism eliminated as such to any extent, like the accompanying purines? What physiological action, if any, can they exert? Are they connected with the metabolism of somewhat related compounds, such as creatinine? An inspection of the structural formulas will suffice to indicate some of these possibilities:

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[4] Ascoli: Zeitschrift für physiologische Chemie, 1900, xxxi, p. 161.

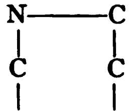
[5] Osborne and Harris: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 109.

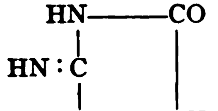
[6] Mandel and Levene: Journal of biological chemistry, 1906, i, p. 425; Zeitschrift für physiologische Chemie, 1906, xlix, p. 264.

[7] Burian: Zeitschrift für physiologische Chemie, 1907, li, p. 438, and earlier papers.

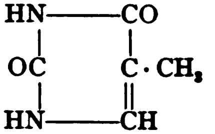
[8] Cf. Steudel: Zeitschrift für physiologische Chemie, 1907, liii, p. 508; Levene and Mandel: Biochemische Zeitschrift, 1908, ix, p. 233.

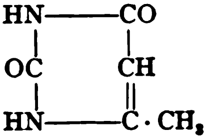
[9] Osborne and Heyl: This journal, 1908, xxi, p. 157.


pyrimidine nucleus


creatinine

Some of the problems have been touched by Steudel.[10] He has attempted to ascertain whether the animal organism is capable of constructing purine derivatives by the addition of a urea group to the pyrimidine skeleton. It must be remembered that some of the artificial syntheses of uric acid and other purines pass through a pyrimidine stage.[11] Steudel's experiments were carried out with dogs — a species not well adapted for the study of the syntheses in question, as this investigator himself admits, because of the readiness with which purines undergo metabolic transformation beyond the uric acid stage (to allantoïn) in the canine organism. In no case could Steudel demonstrate an increased elimination of purines (searched for in the urine as insoluble silver compounds) after feeding daily gram doses of fourteen different pyrimidine compounds. His list included the following.:


thymine


4-methyluracil

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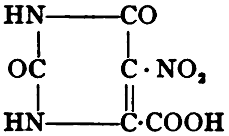
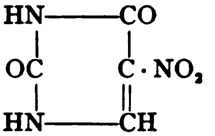
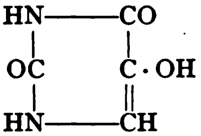
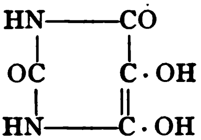
[10] STEUDEL: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 244; 1901, xxxii, p. 285; 1903, xxxix, p. 136.

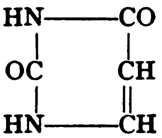
[11] These are discussed by STEUDEL: *Loc. cit.*, xxxix, p. 137.

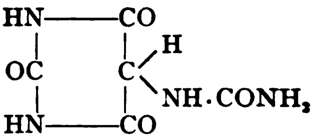

5-nitrouracil-
4-carboxylic acid

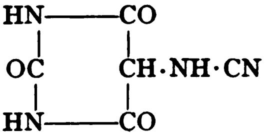

5-nitrouracil

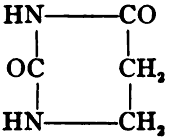

isobarbituric
acid

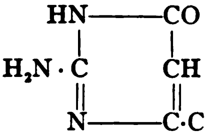

isodialuric acid

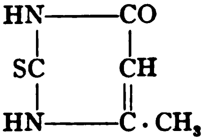

uracil

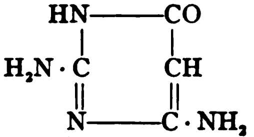

pseudouric acid

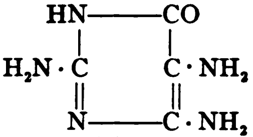

isouric acid


hydrouracil


2-amino-4-methyluracil


2-thio-
4-methyluracil


2-4-diamino-6-oxy-
pyrimidine


2-4-5-triamino-6-oxy-
pyrimidine

Of all these none were recovered in the urine except 4-methyluracil, 5-nitrouracil, 2, 4, 5-triamino-6-oxypyrimidine,[12] and methylsulfouracil. The contrast between 4-methyluracil (recovered) and the closely related 5-methyluracil or thymine (not recovered) is suggestive of an important relationship between chemical structure and susceptibility to metabolic degradation. However, Sweet and Levene[13] have fed thymine in larger doses (6 gm.) to a dog with an Eck fistula and recovered thymine (3.5 gm.) in the urine. They selected an animal in which the liver was excluded, because of the destruction of thymine reported by Steudel for normal dogs.

The excretion of veronal, one of the various pyrimidine derivatives

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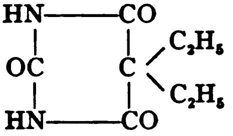
[12] The alleged toxicity of the amino-oxypyrimidines will be discussed in a subsequent paper from this laboratory by Dr. Kleiner.

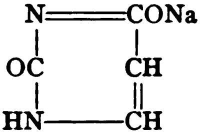
[13] Sweet and Levene: Journal of experimental medicine, 1907, ix, p. 229.



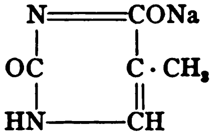
veronal

which have been examined pharmacologically, has been determined by Fischer and von Mering.[14] This compound is eliminated for the most part unchanged by the kidneys. Wolf [15] has tested the action of small quantities of thymine, cytosine, and uracil (35 mgm. per kilo of body weight) on cats, with negative results.

The present paper deals with the fate of thymine, cytosine, and uracil introduced into the organism of man and various animals in different ways.[16] Before undertaking a physiological study of thymine and uracil it seemed desirable to prepare some soluble salts of them which would be suitable for use in this connection; for these three pyrimidines are rather difficultly soluble in water at low temperatures. The preparation of the compounds has been described elsewhere.[17] A potassium salt of thymine has been prepared by Steudel;[18] and the monopotassium salts of both thymine and uracil have been described by Johnson and Clapp.[19] Many physiological chemists refer to thymine and uracil as pyrimidine *bases*. The alkali salts which are described elsewhere clearly demonstrate the acid properties of these two compounds. The sodium salts are the only derivatives satisfactory for



sodium salt of uracil[20]



sodium salt of thymine[20]

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[14] Fischer and von Mering: Therapie der Gegenwart, 1903, v, p. 97; Maly's Jahresbericht für Tierchemie, 1905, xxxv, p. 371.

[15] Wolf: Journal of physiology, 1905, xxxii, p. 171.

[16] The data are taken from the thesis presented by Victor C. Myers for the degree of Doctor of Philosophy at Yale University, June, 1909.

[17] Myers: Journal of biological chemistry, 1910, vii.

[18] Steudel: Zeitschrift für physiologische Chemie, 1900, xxx, p. 539.

[19] Johnson and Clapp: Journal of biological chemistry, 1908, v, p. 59.

[20] The exact constitution of these salts is not known, but it seems probable that the metal would be joined in the most negative position, namely, to the oxygen atom.

[illegible] the pyrimidines can be administered thereby [illegible] all in considerable concentration. A disadvantage [illegible] the alkaline reaction produced by the dissociation of the [illegible] salt. Cytosine was administered in the form of its hydrochloride. We have been fortunate enough to have the advantage of the advice of our colleagues Professors H. L. Wheeler and T. B. Johnson, in the [illegible] preparation of the pyrimidines.

METHODS EMPLOYED.

For the separation of the pyrimidines from the urine the method was [illegible] as follows: In the case of uracil it was simply necessary to precipitate the urine with Hopkins's reagent (mercuric sulphate in acid solution), wash, and disintegrate the precipitate with hydrogen sulphide, boil with charcoal, and repeat the above process. At this point the purines were generally removed with ammoniacal silver solution, and finally the pyrimidine precipitated with silver nitrate and [illegible] washed, decomposed with hydrogen sulphide, boiled with animal charcoal in a solution rendered slightly acid with sulphuric acid to remove barium, filtered and evaporated to small volume. The uracil crystallized out. Thymine and cytosine do not precipitate with mercuric sulphate in acid solution; it was found necessary to render the solution alkaline with potassium or sodium hydroxide in order to precipitate these two pyrimidines. Otherwise the above steps were followed except that in the case of cytosine it seemed feasible to omit the precipitation with silver and baryta; and furthermore, when the solution had been evaporated to small volume, the cytosine was precipitated as the picrate. For the detection of cytosine or uracil, the color reaction of Wheeler and Johnson[22] was of great value, as a few [illegible] of the filtrate, after disintegrating the first mercury precipitate, would allow of their detection. This reaction is as follows: [illegible] of the solution to be tested bromine water is added in slight excess and boiled. The solution is then cooled and bromine water again added until a slight yellow color is produced. An excess of [illegible] produces a deep purple condensation product in the presence of these pyrimidines. The delicacy of this reaction is shown by the fact that 20 mgm. of uracil could be detected in 100 c.c. of human urine in this way. In the case of thymine it was found that where the pyrimidine could be recovered in a very pure form, a characteristic reddish purple diazo reaction could be obtained when applied in the manner described by Johnson and Clapp.[22] This consists in mixing a few milligrams of the dry pyrimidine on a watch glass with an equal proportion of freshly prepared diazobenzenesulfonic acid and then allowing a drop of 10 per cent sodium hydroxide to run on the mixture.

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[22] Wheeler and Johnson: Journal of biological chemistry, 1907, iii, p. 183.

The routine analytical procedures included the Kjeldahl-Gunning method for *nitrogen*, Folin's methods for *urea* and *creatinine*, the Benedict-Myers[23] method for *creatine*, and the Krüger-Schmid[24] method for *purines*.

In the trials on rabbits the bladder was always squeezed out at the same hour each day. The pyrimidines fed were given through a tube.

EXPERIMENTS WITH THYMINE.

Rabbits. *Feeding of sodium thymine.* — I. A rabbit weighing 1.88 kgm. received a uniform daily diet of 300 gm. of carrots. This was

TABLE I.

RABBIT I. THYMINE FEEDING. COMPOSITION OF THE URINE.

Feb. 1909.	Volume.	Sp. gr.	Creatinine.	Total N.	Urea + Ammonia N.	Difference.	Sodium salt of thymine fed.
	c.c.		gm.	gm.	gm.	gm.	
1–2	202	1.019	.068	.703	.606	.097	......
2–3	225	1.017	.092	.607	.500	.107	......
3–4	244	1.014	.082	.605	.466	.149	
4–5	130	1.019	.078	.710	.469	.241	1.0 gm. in 20 c.c. sol. N. = 0.22 gm.
5–6	210	1.017	.082	.882	.547	.335	......
6–7	197	1.015	.089	.577	.431	.146	......
7–8	192	1.015	.088	.541	.446	.095	

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[22] JOHNSON and CLAPP: Journal of biological chemistry, 1908, v, p. 163.

[23] *Cf.* BENEDICT and MYERS: This journal, 1907, xviii, p. 397.

[24] KRÜGER and SCHMID: Zeitschrift für physiologische Chemie, 1905, xlv, p. 1.

completely consumed except on the day of thymine administration, when only 210 gm. were eaten. Since the output of ammonia in rabbits is very small under ordinary circumstances, the urea- and ammonia-N in the urine is reported in total. The protocol is on the preceding page (see Table I).

Creatine was searched for on the day of the thymine feeding and the day following, with negative outcome. From the urine of the fourth day 0.3 gm. of thymine, calculated for the total volume, was recovered. This gave a typical diazo reaction. It was added to the thymine obtained from the urine in the following experiment and Experiment IV, the combined fractions giving a correct analysis. No color reaction could be obtained with bromine water and baryta, indicating that thymine had not been demethylated and thus changed to uracil.

II. A rabbit weighing 2.72 kgm. ate 300 gm. carrots daily, except on the day of thymine feeding, when only 100 gm. were consumed. The protocol follows:

TABLE II.

RABBIT II. THYMINE FEEDING. COMPOSITION OF THE URINE.

Feb. 1909.	Volume.	Specific gravity.	Creatinine.	Total N.	Urea + ammonia N.	Difference.	Total purine N.	Sodium salt of thymine fed.
	c.c.		gm.	gm.	gm.	gm.	mgm.	
12–13	260	1.014	.080	0.796	.710	.086	...	......
13–14	242	1.015	.098	0.835	.733	.102	...	......
14–15	215	1.016	.093	0.877	.755	.122	...	......
15–16	298	1.017	.102	1.207	.921	.266	31.9	2.0 gm. in 50 c.c. sol. N = 0.44 gm.
16–17	77	1.017	.106	0.922	.722	.200	...	
17–18	240	1.016	.101	1.080	.839	.241	...	......
18–19	247	1.013	.102	0.763	.515	.248	...	......

Although the purines were determined on the experimental day only, still the figure obtained shows a slightly increased elimination. Normally, rabbits excrete from 5 to 10 mgm. of purine nitrogen per day on a carrot diet. From 100 c.c. of urine on the fourth day, it was possible to recover 0.250 gm. of almost pure thymine (0.750 gm. calculated for the whole urine). The material crystallized in pure white lustrous leaves and gave a diazo reaction typical only of thymine. The analysis

(Kjeldahl nitrogen) of the combined thymine obtained from this, the preceding, and Experiment IV is given below.

	Calculated for $C_5H_6N_2O_2$:	Found:
N	22.23	22.32

From the urine of the fifth day it was also possible to isolate a small amount of material (0.30 gm. calculated for the total urine) which gave a quite typical diazo reaction.

III. A rabbit weighing 2.62 kgm. ate 300 gm. carrots daily, except on the day of thymine feeding, when 120 gm. were consumed. The chief object of the experiment was to study the influence on the output of purine nitrogen. The protocol follows:

TABLE III.

RABBIT III. THYMINE FEEDING. COMPOSITION OF THE URINE.

March 1909.	Volume.	Specific gravity.	Creat-inine.	Crea-tine.	Total N.	Total purine N.	Sodium salt of thymine fed.
	c.c.	gm.	gm.	gm.	gm.	mgm.	
18–19	235	1.014	.108	.000	0.670	2.6	......
19–20	230	1.012	.106	.005	0.538	3.2	......
20–21	122	1.021	.104	.008	1.455	32.9	2 gm. in 45 c.c. sol. N = 0.44 gm.
21–22	143	1.015	.106	.000	0.825	5.1	......

In this experiment the same results were obtained as those reported in Table II. There was an unmistakable increase in the purine elimination, though small in amount. This was not due to the thymine present in the urine, as the following control experiment shows. In two purine determinations on 300 c.c. samples of normal rabbit urine, to one of which 0.5 gm. of thymine had been added, identical figures were obtained, namely, 4.5 mgm. nitrogen. No attempt was made to obtain the thymine from the urine, as the previous experiments had clearly shown that the thymine could be recovered.

Intravenous injection of sodium thymine. — IV. A rabbit weighing 2.45 kgm. received an injection of 2 gm. of sodium thymine dissolved in 40 c.c. 0.9 per cent sodium chloride solution at 36° C. into the marginal ear vein. No marked symptoms were noted at the time, but the animal suddenly died an hour later. From the urine discharged shortly before death, 0.4 gm. of pure thymine which gave the typical diazo reaction was recovered. (See analysis under rabbit II.)

Dog. *Feeding of thymine.* — Two experiments with thymine were made on a dog. An animal of 6.6 kgm. weight was given 1.0 gm. of thymine mixed with cracker and bone meal. At the end of thirty-six hours 130 c.c. of urine were collected and from this 0.4 gm. of a pure white material, giving the typical diazo reaction of thymine, was obtained. A nitrogen determination on this material, however, gave a low result. To the same dog were given 3.0 gm. of thymine mixed with a small amount of hashed meat. At the end of twenty-four hours 320 c.c. of urine were collected. When precipitated with mercuric sulphate and potassium hydroxide, disintegrated with hydrogen sulphide and boiled with charcoal, 0.509 gm. of almost pure thymine crystallized out. One-half gram more of material was later recovered, making a total of 1.0 gm. When the thymine obtained from the first crystallization was dried, a Kjeldahl nitrogen determination resulted as follows:

	Calculated for $C_5H_6N_2O_2$:	Found:
N	22.23	21.95

Man. *Feeding of thymine.* — It also seemed desirable to determine the action of thymine on man. The subject of the experiment (V. C. M.)

TABLE IV.

MAN. THYMINE FEEDING. VOLUME OF THE URINE.

March 1909.	Volume 10.30 P. M.–10.30 A. M.	Specific gravity.	Volume 10.30 A. M.–10.30 P. M.	Specific gravity.	Volume 24 hr.	Specific gravity.	Creat-inine. gm.	Thymine taken.
25–26	375	1.019	650	1.019	1025	1.019	1.43	....
26–27	300	1.027	820	1.018	1120	1.020	1.50	....
27–28	500	1.017	950	1.014	1450	1.015	1.53	....
28–29	380	1.024	570	1.019	950	1.022	1.49	3.0 gm.
29–30	370	1.024	610	1.019	880	1.022	1.47	....
30–31	325	1.027	570	1.024	895	1.025	1.47	....
31– 1	350	1.024	567	1.025	917	1.025	1.58	....

went without breakfasts and no liquid was taken from 6 P. M. until 12 M., a period of eighteen hours. On this account it was considered that the urine elimination between 10.30 P. M. and 10.30 A. M. would be fairly constant. Such was found to be the case. The day previous to the taking of the thymine was, however, very cold and explains the high volume of the urine on that day. Three grams of thymine were taken at night before retiring. No bad symptoms were noted.

It is obvious, from the data given above, that thymine had no diuretic action. Unfortunately, the subject was not on a purine-free diet and total purine determinations were not made. Thymine appeared to be present in considerable quantity in the urine of the first twelve hours on the thymine day, but it seemed impossible to purify it sufficiently to obtain the typical diazo reaction.

Subsequently the same subject, weighing 62 kgm., remained on a fairly constant, *purine-free* diet. Two and one-half grams of thymine were taken on the fourth day. The determinations of uric acid and purine bases were made in duplicate. The protocol below shows the absence of any detectable influence on the output of these urinary constituents:

TABLE V.

MAN. THYMINE FEEDING. COMPOSITION OF THE URINE.

June 1909.	Volume.	Specific gravity.	Total N.	Creat-inine N	Uric acid N.	Purine base N.
	c.c.		gm.	gm.	gm.	mgm.
18–19	730	1.018	7.50	.52	.103	8.2
19–20	1226	1.012	6.32	.64	.117	10.3
20–21	690	1.018	7.04	.55	.126	11.6
21–22	**460**	**1.026**	**7.29**	**.55**	**.122**	**8.6**
22–23	445	1.027	7.32	.55	.100	8.6
23–24	445	1.026	7.50	.55	.108	10.1

In this experiment no attempt was made to recover thymine from the urine, since the quantity left after the above determinations was too small.

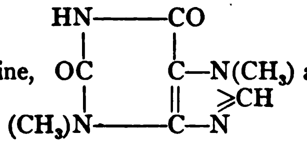
Discussion of the results. — In our experiments with thymine feeding the recovery of the substance unchanged from the urine leaves no doubt of its absorption. The increase in the total nitrogen output in every case after the feeding points to the same conclusion, especially when it is noted in the case of the rabbits that they ate less food than usual on the days in question and thus lowered their food-N intake. The output of creatinine was also not altered in any way.

We have failed, even in the dog, to obtain the complete disappearance of thymine claimed by Steudel. Whether the difference in our results is due to more perfect absorption in our experiments (a point which cannot be determined from Steudel's protocols) or to more perfect methods of isolation employed by us, is not apparent. That the absorption is not rapidly completed is suggested by the occasional lag observed in the output of the urinary thymine, as in Experiment II (Rabbit). The identification of the urinary pyrimidine was satisfactory in every case.

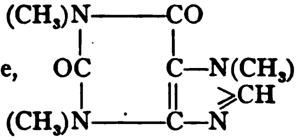
In view of the negative results regarding the synthesis of purines from thymine in the experiment on man, the slight increase noted in the case of rabbits must still be regarded as inconclusive. Sweet and Levene[25] failed to obtain any change in the output of uric acid after feeding 6 gm. of thymine to their Eck-fistula dog. This is, perhaps, not strange if one recalls the capacity of the dog to destroy purines in metabolism; but they likewise failed to obtain an increase in the total nitrogen output, although much of the thymine was recovered as such.

Is thymine a diuretic? — It is a well-known fact that the methylated dioxypurines possess a pronounced diuretic action. In all of those in common use, *e. g.*

theobromine,



and caffeine,



a methyl group is attached to the pyrimidine nucleus. This at once suggests the diuretic possibilities of methylated pyrimidines, such as thymine, or 5-methyluracil. In view of Steudel's claim that thymine is destroyed in the dog's organism, Sweet and Levene[26] utilized animals with Eck fistulæ, believing that with the exclusion of the liver thymine would less readily be destroyed and thus demonstrate any latent diuretic properties. They have reported marked diuresis after addition of 6 gm. of thymine to the purine-free food in one case, and as little as 2 gm. in another. It must be admitted, however, that in view of the marked irregularities in water intake, in their second experiment at least, the data are not so convincing. We quote the protocol (Levene, Table II):

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[25] SWEET and LEVENE: Journal of experimental medicine, 1907, ix, p. 229.

[26] SWEET and LEVENE: *Loc. cit.*; also LEVENE: Biochemische Zeitschrift, 1907, iv, p. 317.

	Water c.c.	Urine c.c.	
March 31	450	350	
April 2	250	150	
April 3	100	150	
April 4	200	100	
April 5	250	150	2 gm. thymine fed.
April 6	500	350	
April 7	270	150	
April 8	125	160	2 gm. thymine fed.
April 9	200	325	
April 10	200	150	

An examination of our own protocols, on the other hand, gives no evidence whatever of any diuresis attributable to the thymine administered either in rabbits or in man. Since the possibility suggested itself that a temporary diuresis might be compensated in a subsequent period and thus escape notice in the record of the volumes of urine excreted during longer periods, the urine was collected directly from the ureters and the rate of secretion measured, in a few experiments on dogs. Protocols are given below:

Rate of urine secretion in dogs after injections of thymine, etc. — I. A dog weighing 15.75 kgm. was anæsthetized with morphia (31 mgm.) and chloral hydrate (ca. 1½ gm.), followed by A. C. E. Cannulas were introduced into the ureters, and the experimental solutions were injected into the facial vein. Control injections were made with alkali solutions equivalent to that used (in molecular proportions) to dissolve the thymine introduced (see Table VI).

II. A second dog, weighing 9 kgm., under chloral hydrate and A. C. E. narcosis, was employed in the same manner as in the previous experiment. One of the ureters was very small, and it was found necessary to employ a short glass cannula in this case. The urine never flowed

TABLE VI.

Time.	Urine flow, left kidney.	Urine flow, right kidney.	Pulse rate per min.	Respiration rate per min.	Nature of the injections.
	c.c.	c.c.			
3.05–10	3.6	1.5	...	...	....
3.10–15	3.6	1.5	132	...	Normal flow
3.15–20	3.6	3.3	...	...	....
3.28–33	2.5	1.0	142	...	....
3.33–38	3.1	1.3	150	35	....
3.42–47	0.7	1.0	140	34	0.3 gm. NaOH in 25 c.c. of 0.9 per cent NaCl. Injected in 2¼ min.
3.47–52	1.3	1.3	130	29	
3.52–57	1.2	1.5	128	28	
4.00–05	0.2	0.8	116	32	1.0 gm. of thymine plus 0.3 gm. NaOH in 25 c.c. of 0.9 per cent NaCl. Injected in 2 min.
4.05–10	0	0.2	104	41	
4.10–15	0	0.1	88	55	
4.15–20	2 drops	0.1	irregular	46	
4.23–28	0	0	142	34	0.1 gm. diuretin in 25 c.c. of 0.9 per cent NaCl plus 3 drops 10 per cent NaOH in 3 min.
4.28–33	0	0	147	57	
4.33–38	0	0	136	56	

regularly from this ureter (left). No urine flowed for several minutes prior to 3.57, but from then until 4.00 three drops flowed. The results are summarized in Table VII.

At five o'clock, after the injection of 0.1 gm. caffeïne, the urine flowed abundantly from the right kidney.

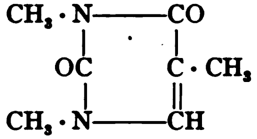
III. A third dog, weighing 8.5 kgm., received 2.0 gm. of chloral hydrate and A. C. E. narcosis. The ureters were both very small, and on this account it was necessary to employ glass cannulas, which could be inserted only a short distance into the ureters. The results are found in Table VIII.

The action of caffeïne shows the susceptibility of the kidney to a suitable diuretic. *No diuresis was obtained with thymine.*

TABLE VII.

Time.	Urine, right kidney, drops.	Urine, left kidney, drops.	Nature of the injections.
4.00–05	8	1	0.3 NaOH in 25 c.c. 0.9 per cent NaCl. 3.57–4.03
4.05–10	3	1	
4.10–15	1	½	
4.17–22	12	¼	1.0 gm. thymine in 25 c.c. 0.9 per cent NaCl + 0.3 gm. NaOH. 4.17–20
4.22–27	1	¼	
4.27–33	2	0	
4.33–38	0	0	0.1 gm. diuretin in 25 c.c. 0.9 per cent NaCl + 0.3 gm. NaOH. 4.33–40
4.38–43	4	0	
4.43–47	8	0	

1, 3-*Dimethylthymine.* — Through the kindness of Professor T. B. Johnson we obtained a specimen of 1, 3-dimethylthymine prepared by Johnson and Clapp.[27] The structure of this compound, which is quite soluble in water, at once suggests possibilities of diuretic action, which were not realized in the only trial made.



1, 3-dimethylthymine

A rabbit weighing 2.46 kgm. received 300 gm. of carrots daily. For several days the urine volume varied from 225 to 250 c.c.; 1, 3-dimethylthymine failed to provoke diuresis, as shown in Table IX.

Experiments with Uracil.

Rabbits. — A rabbit weighing 1.74 kgm. was fed on the usual low-nitrogen carrot diet. Uracil was administered as the sodium salt, pri-

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[27] Johnson and Clapp: Journal of biological chemistry, 1908, v, p. 59.

TABLE VIII.

Time. A. M.	Urine, left kidney, drops (small).	Urine, right kidney, drops (large).	Nature of the injection.
11.05–10	2 per min.	1 per min.	
11.10–15	2 per min.	1 per min.	Normal period
11.15–20	2 per min.	1 per min.	
11.20–30	9	9	0.3 gm. NaOH in 25 c.c. 0.9 per cent NaCl, at 11.18½–20
11.30–40	12	12	0.5 gm. thymine in 25 c.c. 0.9 per cent NaCl 0.15 gm. NaOH at 11.30–31½
11.40–45	0	9	
11.45–50	1	6	
11.50–55	0	9	No apparent symptoms
11.55–60	24	19	0.1 gm. caffeine in 25 c.c. of 0.9 per cent NaCl + 0.15 gm. NaOH at 11.55–56½
12.00–10	72	40	
12.10–15	24	35	
12.15–20	31	20	....
12.20–25	27	16	....
12.25–30	22	24	
12.45–50	17	6	Period before injection
12.50–55	7	8	0.5 gm. thymine in 25 c.c. 0.9 per cent NaCl + 0.15 gm. NaOH at 12.50–51½
12.55–60	0	1	
1.00–05	0	3	

TABLE IX.

May 1908.	Volume.	Specific gravity.	Quantity of dimethylthymine injected.
	c.c.		
23	220	1.025	....
24	293	1.010	
25	242	1.012	....
26	168	1.020	0.13 gm. subcutaneously in sterile solution
27	250	1.013	

marily to determine whether any change in the output of purines would result.

TABLE X.

METABOLISM EXPERIMENT WITH URACIL (FEEDING AND INTRAPERITONEAL ADMINISTRATION). RABBIT I.

Nov. and Dec. 1908.	Volume.	Specific gravity.	Creatinine.	Total N.	Total purine N.	Sodium salt of uracil given.
	c.c.		gm.	gm.	mgm.	
28–29	280	1.014	.063	.420	8.6	....
20–39	208	1.015	.056	.333	10.4	
30– 1	330	1.013	.078	.614	9.6	0.5 gm. *per os* in 40 c.c. sol. N = 0.12 gm.
1– 2	297	1.013	.061	.633	7.7	1.0 gm. *per os* in 20 c.c. sol. N = 0.25 gm.
2– 3	228	1.014	.044	.451	7.9	
3– 4	258	1.014	.066	.534	7.4	0.2 gm. in 4 c.c. sol. *intraperitoneally.* 0.5 gm. *per os* in 20 c.c. solution.
4– 5	228	1.015	.060	.547	9.0	
5– 6	188	1.016	.042	.400	4.5	
6– 7	197	1.015	.042	.325	4.7	....

According to the above data, uracil is without influence on the purine elimination in the urine. The creatinine also remained practically constant. No creatine was found on 1–2. On the third and fourth days an increase in the total nitrogen eliminated is noted, in part proportional to the amount of uracil ingested. No untoward symptoms were noted, nor was the volume of the urine found to be materially changed. The color reaction indicated the elimination of a considerable portion of the uracil. For example, on the third day a moderately strong reaction was obtained in the urine with bromine water and baryta after the usual precipitation. On the fourth day a strong reaction was obtained, on the fifth none, on the sixth a faint reaction, and on the seventh a moderately strong reaction.

Inasmuch as the purine elimination was found to remain unchanged in the first experiment with uracil, it seemed desirable to estimate the urea in a similar experiment. For this experiment the same animal was employed.

TABLE XI.

METABOLISM EXPERIMENT WITH URACIL. RABBIT I.

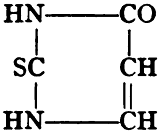
Dec. 1908.	Volume.	Specific gravity.	Creatinine.	Creatine.	Total N.	Urea + ammonia. N.	Difference.	Sodium salt of uracil fed.
	c.c.		gm.	gm.	gm.	gm.	gm.	
8– 9	221	1.013	.036	...	.212	.144	.068	....
9–10	218	1.015	.067	...	.392	.306	.086	
10–11	260	1.011	.030	...	.265	.322	.043	....
11–12	295	1.015	.076	...	.611	.381	.230	1.5 gm. in 35 c.c. sol. N = 0.37 gm.
12–13	182	1.013	.042	none	.360	.281	.079	
13–14	288	1.013	.069	.006	.423	.307	.116	....
14–15	222	1.018	.082	.007	.413	.275	.138	

From the above results there is apparent only a very slight increase in the urea elimination, if any, after the ingestion of uracil. A very strong color reaction was obtained from the urine of the uracil day and a slight reaction from that of the following day. From 100 c.c. of urine on the fourth day, 0.386 gm. of uracil was recovered, which would be equivalent to about 1.15 gm. for the day. After being further purified, it was subjected to a Kjeldahl nitrogen determination.

	Calculated for $C_4H_4N_2O_2$:	Found:
N	25.03	23.9

The uracil was apparently eliminated in a large measure unchanged.

When *2-thiouracil*,[28] in the form of its sodium salt, was administered to a rabbit, the elimination of "neutral" sulphur was greatly increased.



2-thiouracil

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[28] In the synthesis of uracil as described by WHEELER and LIDDLE: American chemical journal, 1908, xl, p. 547, 2-thiouracil is first prepared by condensing thiourea with the sodium salt of formylacetic ester. Upon treating thiouracil with monochloracetic acid uracil results.

In a trial with a guinea pig the urine gave a strong color reaction with bromine water and baryta, after the routine precipitation. It is probable from these data that 2-thiouracil is eliminated, in part at least, unchanged.

Dogs. — Two feeding experiments were made with uracil on dogs. In the first experiment an animal of 6.6 kgm. weight was given 0.5 gm. of uracil mixed with milk and cracker meal. When the 230 c.c. of urine collected on the following morning were precipitated with mercuric sulphate, and the precipitate was disintegrated with hydrogen sulphide, a few cubic centimetres of the 100 c.c. filtrate gave a strong reaction with bromine water and baryta, demonstrating the presence of uracil.

A second experiment was begun on a bitch of 6.9 kgm. weight on January 8. At 10.30 A. M. 3.5 gm. of the sodium salt of uracil in 160 c.c. solution were given to the dog with a tube. A half hour later this was completely vomited up. At 4 P. M. on the same day 2.0 gm. of uracil were given to the dog mixed with hashed meat. The vomitus was dried down and fed the next morning mixed with meat and cracker meal. The first urine was passed on January 10, the 48-hour specimen amounting to 292 c.c. On the next morning (11th), 290 c.c. of urine were collected. One third of the urine from the first two days was precipitated with mercuric sulphate and disintegrated with hydrogen sulphide in the usual manner. The solution now gave a very strong color reaction with bromine water and baryta. After the routine purification, 0.799 gm. of a substance having all of the properties of uracil was obtained. This would be equivalent to 2.4 gm. for the 292 c.c. of urine. After recrystallization the substance gave the following nitrogen determination (Kjeldahl):

	Calculated for $C_4H_4N_2O_2$:	Found:
N	25.03	24.84

A moderate color reaction for uracil was also obtained in the urine of the eleventh.

Man. — It seemed desirable to test the ability of the human organism to destroy uracil and also to determine whether or not any diuresis is produced. The experiment was made in the same way as the one previously described for thymine, the uracil being taken at 11 P. M. just before retiring.

TABLE XII.

METABOLISM EXPERIMENT WITH URACIL. MAN.

March, 1909.	Volume 10.30 P. M.–10.30 A. M.	Specific gravity.	Volume 10.30 A. M.–10.30 P. M.	Specific gravity.	Volume 24 hr.	Specific gravity.	Creatinine.	Uracil taken.
	c.c.		c.c.		c.c.		gm.	
6– 7	395	1.015	590	1.029	985	1.025	1.57	....
7– 8	445	1.020	594	1.023	1039	1.022	1.56	....
8– 9	475	1.022	775	1.014	1250	1.016	1.69	....
9–10	455	1.019	700	1.019	1155	1.019	1.70	2.0 gm.
10–11	310	1.025	485	1.025	795	1.025	1.50	....
11–12	330	1.028	656	1.018	986	1.020	1.60	....
12–13	350	1.024	750	1.019	1100	1.020	1.59	....

No untoward symptoms were noted, and uracil apparently had no specific influence on the volume of the urine. From the first twelve hours' urine on the uracil day, a moderately strong color reaction was obtained after disintegrating the mercury precipitate. Five cubic centimetres of a total volume of 250 c.c. were employed for this test. However, sufficient uracil could not be separated for a nitrogen determination. No color reaction could be obtained in the urine for the second twelve hours.

Discussion of the results. — No toxic symptoms were obtained in any case with uracil. The output of purines, in the rabbit, remained unaltered by uracil, which was evidently absorbed. The elimination of creatinine was not notably disturbed, nor was any diuresis noted beyond what might be attributed to the water of solution introduced. Our experiments again differ from the conclusions of Steudel, who claims that uracil disappears in the organism of the dog. We have succeeded in isolating the excreted compound and noting the rate at which its elimination proceeds.

EXPERIMENTS WITH CYTOSINE.

Since Steudel[29] has suggested that the amino pyrimidines may be toxic, preliminary trials were made on *guinea pigs* to determine this.

Guinea pigs. — An intraperitoneal injection of 0.4 gm. of cytosine hydrochloride in about 4 c.c. of solution was given to a guinea pig of 290 gm. weight. Several hours later, about 5 c.c. of urine were passed. The urine itself gave an intense color reaction with bromine water and baryta. An equally intense reaction was obtained from the urine on the next morning, but no reaction could be obtained from the urine on the third day. The animal died on the fourth day; but an autopsy indicated that death was very probably due to peritonitis.

Two tenths of a gram of cytosine hydrochloride were injected subcutaneously in the back of a guinea pig of 500 gm. weight. The urine itself on the next morning did not give the color reaction, but, after the routine precipitation with mercury, an intense reaction was obtained. No test for cytosine could be obtained in the urine on the succeeding day.

An experiment was made to determine the delicacy of the color reaction and also to determine whether or not a small amount of cytosine hydrochloride would be destroyed. To the same guinea pig 50 mgm. of cytosine hydrochloride were given *subcutaneously*. From the urine collected on the next morning a distinct color reaction could be obtained.

Two days later 1.0 gm. of cytosine hydrochloride in about 5 c.c. solution was given *subcutaneously* in the back of the same animal. After the injection a few convulsive symptoms were noted, but these quickly disappeared. On the next morning the guinea pig appeared in good condition. The urine itself gave a slight color reaction, and, after the routine precipitations with mercury and silver, a very strong reaction. When the recovered material was evaporated to dryness and treated with a few cubic centimetres of dilute hydrochloric acid, it immediately went into solution, showing the absence of uracil and hence that cytosine had not been deamidized in the body. The weight of the pure white residue obtained after evaporation was 0.950 gm. From this only 0.150 gm. of cytosine picrate could be obtained. A nitrogen determination (salicylic acid modification of the Kjeldahl-Gunning method) resulted as follows:

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[29] STEUDEL: Zeitschrift für physiologische Chemie, 1901, xxxii, p. 290.

	Calculated for $C_4H_5ON_3 \cdot C_6H_2(NO_2)_3OH$:	Found:
N	24.63	24.46

No color reaction could be obtained from the urine on the next morning. Notwithstanding the large amount of material injected in such a small animal, no marked symptoms were observed. The animal was apparently in the best condition several weeks later. *Cytosine is evidently non-toxic.*

Rabbits. — Two metabolism experiments were made on rabbits. In the first of these the cytosine was fed and in the second injected. A rabbit of 2.52 kgm. weight on the usual carrot diet was used in the first experiment.

TABLE XIII.

METABOLISM EXPERIMENT — CYTOSINE PER OS. RABBIT.

Feb 1909.	Volume.	Sp. gr.	Creatinine.	Creatine.	Total N.	Urea + ammonia N.	Difference.	Cytosine hydrochloride fed.
	c.c.		gm.	gm.	gm.	gm.	gm.	
17–18	240	1.016	.101	.014	1.080	.839	.241	....
18–19	247	1.013	.102	.017	0.763	.515	.248	....
19–20	228	1.016	.107	.000	0.855	.607	.248	
20–21	270	1.011	.101	.006	0.846	.567	.279	2.0 gm. in 35 c.c. sol. N = 0.56 gm.
21–22	262	1.014	.095	.008	0.751	.550	.201	
22–23	255	1.013	.103	.005	0.830	.528	.302	....
23–24	222	1.007	.090	.005	0.573	.393	.180	

No particular changes were observed in the various urinary constituents as being due to the ingestion of cytosine. The urine of both the fourth and fifth days gave a strong color reaction with bromine water and baryta. It was possible to isolate from the two urines, calculated for their total volumes, 1.0 gm. of cytosine picrate, about 0.15 gm. being from the fifth day. After the removal of the cytosine it was impossible to detect uracil. The dry residue, both before and after removing the cytosine, immediately went into solution upon treating with a few cubic centimetres of dilute hydrochloric acid, which would not have been the case if uracil had been present. Wheeler and Johnson[30]

[30] WHEELER and JOHNSON: American chemical journal, 1903, xxix, p. 492.

have shown that cytosine picrate has a melting-point of 264° C., unless absolutely pure, and this was found to be the case here. A nitrogen determination (salicylic acid modification of the Kjeldahl-Gunning method) resulted as follows:

	Calculated for $C_4H_5ON_3 \cdot C_6H_2(NO_2)_3OH$:	Found:
N	24.63	24.78

The second experiment was made upon an animal of 2.44 kgm. weight on the usual carrot diet, the cytosine hydrochloride being given intravenously in the marginal vein of the ear. It was dissolved in 30 c.c. of 0.9 per cent sodium chloride solution and injected slowly during fifteen minutes at a temperature of 36° C. In this animal a study was likewise made of the purine elimination.

TABLE XIV.

METABOLISM EXPERIMENT — CYTOSINE INTRA VENAM. RABBIT.

March 1909.	Volume.	Specific gravity.	Creat-inine.	Crea-tine.	Total N.	Total purine N.	Cytosine hydrochloride intravenously.
	c.c.		gm.	gm.	gm.	mgm.	
6– 7	262	1.014	.168	.032	1.194	8.4	
7– 8	326	1.010	.121	.016	.538	4.4	
8– 9	215	1.015	.104	.022	.571	3.4	
9–10	289	1.008	.107	.016	1.088	2.6	1.5 gm. in 30 c.c. solution N = 0.42 gm.
10–11	206	1.012	.106	.015	.797	1.9	
11–12	207	1.010	.100	.008	.621	1.8	
12–13	235	1.012	.102	.012	.634	3.3	
13–14	220	1.009	.100	.009	.574	3.6	

A short time after the injection the rabbit was found eating, and no bad symptoms were observed. On the day the cytosine was given an increase was observed in the total nitrogen elimination in proportion to the amount of cytosine injected. The purine elimination, however, was not increased. In this urine a very strong color reaction was obtained with bromine water and baryta after the routine precipitation with mercury and disintegration with hydrogen sulphide. Only the merest trace of purine precipitate could be obtained in the urine of this day. From half the urine 0.355 gm. of cytosine picrate was obtained, equivalent to 0.71 gm. for the total urine. The picrate was crystallized from a hot solution and the needles were a half-inch in length. The usual nitrogen determination resulted as follows:

	Calculated for $C_4H_5ON_3 \cdot C_6H_2(NO_2)_3OH$:	Found:
N	24.63	24.67

No color reaction could be obtained in the urine of the fifth day, showing that the cytosine was speedily eliminated after injection.

Dog. — One injection experiment with cytosine hydrochloride was made with a dog. An animal of 10.6 kgm. weight was given an intraperitoneal injection of 2.0 gm. of cytosine hydrochloride in 15 c.c. of physiological saline at 11 A. M. March 27. The next morning, at 10.30, 205 c.c. of urine were collected. Half of this urine was precipitated with mercury in the usual manner, disintegrated with hydrogen sulphide, and boiled with animal charcoal. A few cubic centimetres of this filtrate gave an intense color reaction with bromine water and baryta. The mercury precipitation was repeated as above, the filtrate precipitated with silver nitrate and baryta, disintegrated with hydrogen sulphide, and boiled with animal charcoal. From this 0.105 gm. cytosine was obtained as the picrate, an equivalent of 0.215 gm. for the whole urine.

On the morning of the 28th the dog was fed its customary meal and on the 29th at 9 A. M. 111 c.c. of urine were collected. No cytosine could be detected in the urine.

The usual nitrogen determination on the 0.105 gm. of cytosine picrate resulted as follows:

	Calculated for $C_4H_5ON_3 \cdot C_6H_2(NO_2)_3OH$:	Found:
N	24.63	24.68

Man. — An experiment was made to ascertain the ability of the human organism to destroy cytosine and also to determine the influence, if any, on the quantity of urine secreted. The procedure employed was the same as in the case of thymine and uracil. The cytosine hydrochloride was taken at 10.30 P. M. just before retiring. No symptoms of any kind were noted.

TABLE XV.

March, 1909.	Volume 10.30 P. M.–10.30 A. M.	Specific gravity.	Volume 10.30 A. M.–10.30 P. M.	Specific gravity.	Volume 24 hr.	Specific gravity.	Creat-inine.	Cytosine hydro-chloride taken.
	c.c.		c.c.		c.c.		gm.	
11–12	330	1.028	656	1.018	986	1.020	1.60	....
12–13	350	1.024	750	1.019	1100	1.020	1.59	....
13–14	300	1.027	1165	1.013	1465	1.015	1.52	....
14–15	295	1.027	425	1.022	720	1.025	1.58	2.0 gm.
15–16	320	1.027	485	1.025	705	1.026	1.43	....
16–17	350	1.025	955	1.015	1305	1.017	1.68	....
17–18	355	1.024	500	1.025	855	1.025	1.54	....

The twelve-hour urines between 10.30 P. M. and 10.30 A. M. are remarkably constant in volume throughout. The normal constancy is also noted in the creatinine elimination. On the day the cytosine was taken a strong color reaction was obtained with the urine of the first twelve hours, and a moderate reaction in that of the second twelve hours. Although a strong color reaction was observed, only 0.15 gm. of cytosine picrate could be isolated from the first twelve hours' urine. No uracil could be detected. A nitrogen determination was made in the customary manner with the following result:

	Calculated for $C_4H_5ON_3 \cdot C_6H_2(NO_2)_3OH$:	Found:
N	24.63	24.70

Discussion of the results. — Cytosine hydrochloride is not toxic according to the evidence obtained on various species in our experiments. It does not alter the output of purines under conditions where it is obviously absorbed, nor does it alter the output of creatinine. It has no specific diuretic properties. Cytosine was recovered unchanged in the urine of all types of animals investigated. Uracil could not be obtained as a product of deamination of cytosine in metabolism.

EXPERIMENTS WITH NUCLEIC ACIDS.

In their study of nuclein metabolism in a dog with Eck's fistula, Sweet and Levene[31] were unable to find thymine in the urine when nucleic acid was fed, although, as already mentioned, thymine ingested as such was excreted again. They concluded that either nucleic acid is not completely disintegrated in the organism or is metabolized so slowly that the thymine liberated has a chance to be destroyed.

Our experience in a few experiments has been similar to this. Ten grams of yeast nucleic acid (from Parke, Davis & Co.) were fed in the form of sodium nucleate through a stomach tube to a rabbit weighing 2.3 kgm. The pyrimidines which are obtained from yeast nucleic acid, cytosine and uracil, both give the color reaction with bromine water and baryta. On this account it was thought that if either of these pyrimidines passed into the urine, they could easily be detected. However, neither on the day following the nucleic acid feeding nor on the succeeding day could a color reaction be obtained after the usual mercury precipitation.

An attempt was made to detect cytosine in the urine of two men receiving a rich nucleoprotein diet, by means of the color reaction of Wheeler and Johnson. These individuals eliminated 1.0 gm. and 0.7 gm. of uric acid respectively. Several hundred cubic centimetres of urine from both were twice precipitated with mercuric sulphate and potassium hydroxide, the precipitate disintegrated with hydrogen sulphide, and the resultant solution concentrated. No color reaction for the pyrimidines could be obtained.

Further trials in this direction are proposed before any final conclusion is drawn from such data. It will be necessary, first of all, to feed nucleic acids in quantities yielding an amount of pyrimidines comparable with what is known from our experiments to reappear in the urine.

EXPERIMENTS WITH PYRIMIDINES AND LIVER EXTRACTS.

Reference has already been made to the possibility of the existence of tissue enzymes capable of producing in the pyrimidines changes analogous to those experienced by purines. Aside from the well-known enzymatic deamination reactions and the oxidative changes, a demethylation of trimethylxanthine (caffeïne) by means of tissue extracts has been reported by Kotake[22] and by Schittenhelm.[23] We desire to record protocols of a few trials with extracts made from fresh livers of pigs. The hashed tissues were extracted with 3–4 volumes of water, allowed to stand forty-eight hours, and filtered.

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[31] SWEET and LEVENE: *Loc. cit.*

Thymine. — For thymine, 500 c.c. of liver extract and 0.5 gm. of thymine were employed. They were allowed to stand at room temperature with toluene in a closed vessel for six weeks. At the end of that time no color reaction could be obtained for uracil, and though the work was not quantitative, the thymine could in a large measure be recovered. The method was as follows:

The protein remaining in solution was removed by coagulation. The filtrate was precipitated with mercuric sulphate and potassium hydroxide, and the precipitate disintegrated with hydrogen sulphide, boiled with animal charcoal, and the process repeated. The purines were now removed with ammoniacal silver solution, and the pyrimidines finally precipitated with silver nitrate and baryta after slightly acidifying the above solution with nitric acid. The precipitate was filtered off, washed, slightly acidified with sulphuric acid to remove barium, and disintegrated with hydrogen sulphide. The solution was cleared with animal charcoal, filtered and concentrated. No color reaction could be obtained with bromine water and baryta, but thymine crystallized out; hence it is assumed that thymine had not been demethylated and changed to uracil.

Cytosine. — The same procedure was employed for cytosine, 0.5 gm. of the hydrochloride being used for 500 c.c. of liver extract. From the silver and baryta filtrate a strong color reaction was obtained with bromine water and baryta, and when picric acid was added to the solution, cytosine picrate at once crystallized out, showing that the bulk of the cytosine at least had remained unchanged.

Uracil. — Two series of experiments were made to determine whether or not enzymes capable of destroying uracil were present in the liver. To 500 c.c. of fresh liver extract, 0.3 gm. of the sodium salt of uracil was added. Four autolyses were made, all in the presence of toluene; in (*a*) air was allowed to bubble through eight hours per day; (*b*) was closed; in (*c*) air was allowed to bubble through boiled extracts; (*d*) was a control with air, but no uracil. In both series of experiments air was allowed to bubble through the extract as indicated for ten days in a thermostat at 38° C.; but at the end of that time the color reaction with bromine water and baryta could always be obtained in the solution to which uracil had been added. The liver extracts were "active," as the changes in the purines showed. However, the details of the latter are hardly of sufficient importance to be reported here.

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[22] Kotake: Zeitschrift für physiologische Chemie, 1908, lvii, p. 378.

[23] Schittenhelm: Zentralblatt für Stoffwechsel, 1908, iii, p. 290.

The above data indicate that in the pig liver, at least, no enzymes are present which are easily capable of producing extensive changes in the pyrimidines under the conditions of our trials.

CONCLUSIONS.

Our experiments have been carried out on normal animals under conditions which leave no doubt regarding the actual introduction of the pyrimidines used into the circulation. It appears that neither cytosine nor uracil contributes to the formation of the purines eliminated in the urine. The same conclusion may be applied to thymine if one excepts two experiments on rabbits in which a slight increase of purine elimination was noted. It is unlikely that this was due to a synthesis from the thymine introduced, since the transformation would require a demethylation of thymine for which we have as yet established no evidence. An indirect effect is equally plausible as an explanation in these instances. The creatinine metabolism was unaffected by any of the compounds used.

The experiments with the three pyrimidines introduced as such into the organism indicate that they are more stable in metabolism than are the familiar purines, which readily undergo transformation.[34] Contrary to the experiments of Steudel, who has devoted much attention to the pyrimidines, *we were able to recover cytosine,*[35] *uracil, and thymine from the urine of various species* in feeding and injection trials. The absence of marked increase in the urea-ammonia output, despite the increased elimination of nitrogenous compounds, likewise speaks against extensive conversion to urea. On the other hand, pyrimidines have not yet been detected in the normal urine even after feeding nucleic acids.

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[34] Extensive data on the behavior of the purines in metabolism have been collected in this laboratory by Dr. J. F. LYMAN and will soon be published.

[35] No data on cytosine have been reported by others.

Whether this is the result of quantitative conditions or more specific metabolic causes is not clear. One recalls the curious reverse anomaly in cystinuric individuals who are unable to destroy the endogenous cystine, but oxidize the same product when it is fed to them.

The pyrimidines studied are devoid of marked pharmacological properties. Neither thymine nor dimethylthymine exhibits any diuretic action in normal animals or man. Cytosine, an aminopyrimidine, shows no marked toxicity. Further details have been discussed in connection with the experimental data.

THE PHYSIOLOGY OF CELL-DIVISION. — II. THE ACTION OF ISOTONIC SOLUTIONS OF NEUTRAL SALTS ON UNFERTILIZED EGGS OF ASTERIAS AND ARBACIA.

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I. INTRODUCTORY.

THE conditions under which cell division and development may be initiated in unfertilized eggs are now known to be both numerous and highly varied.[1] Temporary alteration of the osmotic equilibrium between the egg and its medium (action of hypertonic and hypotonic[2] solutions), temporary exposure to high (34° to 38°) or low (near freezing) temperatures, mechanical or electrical treatment,[3] the action of numerous electrolytes, coagulative substances,[4] and fat solvents (including bile salts), and, finally, the action of certain (chiefly hæmolytic) alkaloids (saponin, solanin, digitalin) and foreign blood sera, have all been found, either alone or in various combinations, to produce this effect. For the greater part of the experimental progress in this fundamental field of research and for a most suggestive and far-reaching analysis of the nature of the initiatory process, biological science is indebted to the work of Jacques Loeb, to whom all investigators in this and related fields must acknowledge profound obligations.

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[1] It is impossible within the limits of this article to review the literature of artificial parthenogenesis. Professor LOEB's recent book "Die chemische Entwicklungserregung des tierischen Eies," Berlin, 1909, contains an exhaustive survey of the subject.

[2] MCCLENDON reports having started cleavage in sea urchin eggs by temporary exposure to dilute sea water: "Science," 1909, p. 454.

[3] *Cf.* DELAGE: Archives de zoologie expérimentale et générale, 1908, sér. 4, ix, notes et revue, p. xxx.

[4] DELAGE's experiments with tannin solutions containing ammonia belong to this category. This method also gives good results at Woods Hole with Arbacia. I have tested the action of several protein precipitants — tannin, picric acid, phosphotungstic acid, in concentrations of $\frac{1}{25}$ per cent and $\frac{1}{50}$ per cent in sea water — on starfish eggs, and have found that membrane formation, cleavage, and in some cases (especially with tannin) development to the blastula stage may be induced by brief exposure (varying from one to ten minutes) to these solutions.

The facts of artificial parthenogenesis have shown clearly that in each unfertilized resting egg there is inherent a latent or quiescent developmental mechanism which may be set in operation by any one of a variety of external changes of condition; they also indicate that this mechanism once set in motion tends to run its course automatically, although it may be checked or diverted by abnormal external or internal influences. The essential and theoretically significant facts established by the above researches are, thus, (1) that a temporary initial change of condition may cause the germ to pass through the entire cycle of developmental transformation, and (2) that the exact nature of this initial change is a matter of secondary importance.

In the development of most metazoa mitotic cell divisions — in the early stages at least — appear to form the chief agency in the orderly transformation and distribution of material; typically cell division is the first evident change in the developing egg, and apparently the initiation of cleavage in some way involves the initiation of development. Just why development should be almost universally associated with mitotic cell division is a problem whose consideration I shall not attempt in the present paper. It is sufficiently clear that further insight into the physical and chemical nature of this complex process — or rather combination of processes — will be essential for a fuller understanding of development. In the present paper I propose to consider cell division by itself, disregarding provisionally its relation to development; and I shall treat the problem of the initiation of development as if it involved merely the simpler problem of the initiation of cell division. The nature of the initial change in cell division is thus the chief question to be considered. The rhythm of division, once the process is started, tends to continue; that it is accompanied by progressive embryonic differentiation may for the present be regarded simply as an incidental complication.

First, it is to be noted that the production of a common effect, namely, the initiation of cell division, under the influence of the above diverse conditions, suggests that the same fundamental and critical change in the egg is primarily induced by all of these various forms of treatment, and that the succeeding cleavage, with its characteristic attendant phenomena, is a consequence of this primary change. In this respect a distinct analogy is shown to the general process of stimulation, in which also a definite and qualitatively constant effect, *e. g.*, muscular contraction, is produced by a wide diversity of agencies. It is in fact noteworthy that many of the agencies that excite cell division in unfertilized ova have also a stimulating action on muscle or nerve. This agreement implies the possibility that the primary change in the two widely dissimilar processes is of the same essential nature. Now the primary change in stimulation, as many facts of the most diverse kind indicate, is almost certainly an increase in the permeability of the plasma membrane. If the resemblance between stimulation and the initiation of cleavage is more than a superficial one, or one suggested by a merely verbal analogy, we should therefore expect to find evidence that in the latter case also an increase in the permeability of the plasma membrane is the critical or determining factor in the initiation of the process.

Abundant and in my opinion almost demonstrative evidence exists that the primary change in stimulation is an increase in the permeability of the plasma membranes of the irritable elements.[5] There is also evidence, though less direct, that a periodic change of a similar kind occurs in dividing cells.[6] If this is the case, it might be expected that an artificially induced increase in permeability would start the rhythm of division in resting cells, such as unfertilized ova. A visible surface change, frequently connected with a secretion or separation of material from the egg, is in fact a wide-spread phenomenon in normal fertilization, and seems to indicate a primary increase in permeability.[7] The internal processes in cell division are, however, of a more obvious kind, and have attracted the main interest of biologists, so that the possibility that a surface change might have a determinative relation to the whole complex chain of events has been for the most part disregarded, and

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[5] I have summarized this evidence in "Science," 1909, pp. 245–249. *Cf.* also This journal, 1909, xxiv, p. 14.

[6] *Cf.* my article "The general biological significance of changes of permeability" in Biological bulletin, 1909, xvii, pp. 201 *seq.*

[7] Besides the formation of definite fertilization membranes, which occur in relatively few groups (Echinoderms, certain Mollusca, Amphioxus), there are instances of definite secretion of materials present in the surface layer of the unfertilized egg (certain Annelids), and numerous instances of surface tension changes.

[illegible]

lipoids and proteins in which the two components are in some form of intimate union, possibly chemical combination; in cytolysis this union is dissolved by the action of the lipoid-solvent or lipoid-combining alkaloid (as saponin); the proteins then absorb water, and the cell swells and disintegrates. This view of the mechanism of cytolysis is supported by the observations of Knaffl-Lenz.[10] Such cytolytic action, if confined to the surface layers of the egg, leads simply to membrane formation, which in some unknown manner — presumably by indirectly furthering oxidations — initiates in its turn the cleavage process.

A connection between cytolytic action and the initiation of cleavage appears to me to have been clearly and finally demonstrated by Loeb's experiments. Such a condition, however, is naturally open to more than one interpretation. It is evidently in harmony with the above view that an increase of permeability is the primary change in cell division. A prevalent theory of the mechanism of cytolysis is, in fact, that this process is a direct consequence of a pronounced increase in the permeability of the plasma membrane, sufficient to abolish the normal semi-permeability. This is the view upheld by Hamburger, Gryns, Overton, Koeppe, Höber, and many other physiologists. Osmotic equilibrium is thus destroyed, the cell absorbs water, and its soluble constituents diffuse into the surrounding medium, with the necessary result that protoplasmic disintegration soon follows. It is clear that loss of the normal permeability is one actual consequence of the cytolytic process, since the pigment and other protoplasmic constituents do in fact diffuse from the cell. If the above view is correct, the primary and essential action of a cytolytic substance is simply to increase the surface permeability of the cell; the absorption of water and the succeeding protoplasmic dissolution are secondary consequences which follow if the loss of normal semi-permeability is complete and prolonged.

Evidently the increase in permeability may vary in duration and in degree; if relatively slight and transient, it may lead to no evident disintegration and yet produce indirectly a profound alteration in the chemical activities of the cell, *e. g.*, through the loss of certain substances, or the entrance of others from the outside, or quite possibly simply through an accelerated liberation of carbon dioxide with consequent increase in the rate of intracellular oxidations. The conditions of chemical equilibrium within the cell can hardly fail to be modified by even a brief increase in permeability, and the changes in metabolism resulting from this increase may well find their expression in the initiation of the mitotic process. It is clear, as Loeb has repeatedly emphasized, that what is to be accounted for is the alteration in the character of cell metabolism; in referring this to an increase of surface permeability, as in the present hypothesis, I am simply applying to the case of cell division the theory which I have advocated at length for the stimulation process in muscle, where also oxidative cell metabolism undergoes a marked increase apparently in consequence of a sudden increase in surface permeability. The resemblance between the conditions of stimulation in this tissue and those of the initiation of cleavage in unfertilized eggs is thus, on the present view, not merely an analogy, but is based on the actual identity of the primary determining change.

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[10] KNAFFL-LENZ: Archiv für die gesammte Physiologie, 1908, cxxiii, p. 279. For a similar conception of protoplasmic structure *cf.* V. PROWAZEK: Biologisches Centralblatt, 1909, lxix, p. 784.

This view is obviously quite consistent with Loeb's hypothesis of a superficial cytolysis as the primary event in the initiation of cleavage, since such a change could scarcely fail to increase the permeability of the surface layers. Loeb, however, in his numerous papers on artificial parthenogenesis, nowhere attributes a critical significance to the associated changes of permeability; and it is to be assumed that, consistently with his views on cell permeability in general, he regards them as of minor importance. It is true that more recently in the preface to his book, "Chemische Entwicklungserregung," he has made the interesting suggestion that an increased surface permeability may facilitate the entrance of oxygen and hydroxyl ions (or possibly other substances) into the egg,[11] and so further oxidations. Now it is clearly a matter of some importance to decide whether the initial change in *permeability* is in itself of critical moment, or is merely accessory to other and more important changes. Further research alone can decide this question finally; yet certain general considerations bearing on the whole vexed question of cell permeability appear to shed some light on the matter, and I shall therefore briefly present these before proceeding to the descriptive part of this paper.

The importance which a student of fundamental physiological problems will attach to changes of permeability must depend on his conception of what the normal conditions of permeability are in the living resting cell. On this question the views of leading physiologists are still at variance. Loeb has assumed in all of his papers on ion action that dissolved substances such as neutral salts are quite free to diffuse into or out of living cells; on this view such phenomena as plasmolysis require for their explanation no other assumption than that the cell boundary is less freely permeable to such dissolved substances than to water.[12] Overton, on the contrary, has consistently maintained that an absolute impermeability to the diffusion of most crystalloid substances (other than those soluble in lipoids) is an essential characteristic of living cells; this does not mean that such substances are unable to enter or leave cells, but merely that in their entrance or exit active processes other than simple diffusion are concerned. Decision between these contrasted views is rendered more difficult by the circumstance that the permeability of cells readily undergoes change, so that under experimental conditions it is often uncertain whether the observed permeability is that normal to the living cell or is induced artificially.[13] If, however, the cell in its normal condition is enclosed by a membrane impenetrable to the diffusion of most dissolved substances in its interior or environment, it is evident that an increase of permeability must be an event of more critical importance than if the membrane were already permeable.

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[11] *Loc. cit.*, p. xvi.

Various considerations, besides such facts as the high and constant turgor of living plant cells and those adduced by Overton, Hedin, Gryns, and Hamburger, appear to me strongly to favor the latter of the above two opposed views. If non-permeability to the diffusion of the above crystalloids is, as Overton maintains, a characteristic of all living cells, some fundamental significance must be attributed to this peculiarity. This significance I believe to be the following: briefly, *such non-permeability is the main condition on which depends the preservation of the chemical organization of the cell;* on this view what has seemed paradoxical to many biologists — that the cell should be enclosed by a membrane impermeable to the diffusion of necessary salts and food constituents — becomes at once intelligible as an indispensable condition of the continued existence of the complex living system in its usually widely dissimilar environment. The diffusion of soluble substances *from* the protoplasmic complex is prevented; hence the cell is enabled to preserve its normal chemical constitution, with the associated vital properties, independently — within certain limits — of the character of its surroundings. The difficulty of the alternative view is not removed by assuming that soluble and diffusible constituents are held in the protoplasm in some form of combination or by adsorption. In adsorption equilibria are involved, as the characteristic equation indicates: *i. e.*, the presence of a considerable quantity of a soluble substance in the adsorbed and presumably non-diffusible state requires also its presence in a free and diffusible state in sufficient concentration to maintain the equilibrium. Again, the chief colloidal and water-insoluble constituents of the protoplasm are readily hydrolyzable, and the conditions for their hydrolysis undoubtedly exist in all living cells; hence it can scarcely be doubted that the protoplasmic complex contains free amino-acids and sugars; it must also be assumed that at least part of the inorganic constituents exist in the form of ions free to diffuse, even though another part may be in combination, as ion proteids or otherwise. If these constituents were free to diffuse from the cell, it is evident that the existence of any stable chemical organization would be precluded, and the delicate balance of conditions on which the maintenance of normal life processes depends would be impossible. We are thus forced to the conclusion either that the living protoplasm constantly exercises a counteracting activity to prevent its own disorganization by outward diffusion of soluble constituents, or else that its boundary surface is impenetrable — except under special and temporary conditions — to such diffusion. It seems preferable to proceed on the second and simpler alternative.[14] Obviously on this view the intake of food materials, as also the separation of definite substances in secretion, must be due to a special physiological activity of the cell, in which physical diffusion is a subordinate factor; and there is sufficient evidence that this is in fact the case.

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[12] J. Loeb: Oppenheimer's Handbuch der Biochemie, 1908, ii, p. 105; Dynamics of living matter, p. 41, *etc.*

[13] This uncertainty is the main source of the disparity between Loeb's and Overton's results and conclusions.

We conclude, therefore, that the theory of an impermeable plasma membrane in Overton's sense is well founded. It follows from the above considerations that loss of the vital semi-permeability must involve the loss of diffusible materials from the cell; of this the exit of pigment in hæmolysis or cytolysis is a visible instance; the more diffusible cell constituents are known to leave the cell in hæmolysis before the colloidal hæmoglobin.[15] Loss of the physiological polarization is a second consequence which apparently involves coagulative changes in the protoplasmic colloids.[16] The eventual result is disorganization and death of the cell; it has in fact long been recognized that cell death is everywhere associated with a marked increase of surface permeability. The whole evidence thus seems clearly to indicate that the living condition is incompatible with more than a temporary loss of semi-permeability. Permanent loss of this property involves in itself the destruction of cell organization.

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[14] *Entia non sunt multiplicanda præter necessitatem!* The former alternative is of course possible; but such a condition, if existent, would in effect be equivalent to impermeability of the boundary layer, though the conditions of this impermeability would be more complex than the second and simpler view assumes.

This view implies, on the other hand, that the normal permeability of the living cell may be *decreased* without injury, as appears to be the case in the various forms of physiological inhibition, narcosis, and anæsthesia. During these states stimulation, which involves an increase of permeability to a critical degree, is rendered difficult or prevented, and gaseous interchange is checked with resultant lowering of oxidative metabolism. These phenomena, with others, indicate a decreased permeability as their common basis.[17] The partial suspension of physiological activities in inhibition or anæsthesia thus depends on an alteration of permeability in a direction the inverse of that associated with stimulation and with the death process. The reversibility of anæsthesia, as contrasted with the irreversibility of the death process, becomes intelligible on this hypothesis. The contrast depends essentially on the opposite nature of the permeability changes with which the two processes are respectively associated. Activities suspended through a decrease in the permeability of the plasma membrane require for their renewal no more than a return of this property to the normal, since there is no loss of essential materials from the cell, and hence no permanent injury. On the other hand, it is at once evident — since diffusion is from regions of higher to those of lower concentration — that a cell which has lost its essential diffusible constituents through a prolonged increase of permeability cannot regain its original composition and properties through a mere return of the plasma membrane to its normal condition. An irreversible disorganization or death is thus the inevitable consequence of prolonged loss of semi-permeability.

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[15] *Cf.* G. N. Stewart: Journal of physiology, 1901, xxvi, p. 470.

[16] R. S. Lillie: This journal, 1908, xxii, pp. 81, 82.

[17] For evidence that anæsthesia and inhibition are associated with a decrease of permeability *cf.* my paper in this journal, 1909, xxiv, p. 14. The characteristic lowering of irritability in anæsthesia need not in itself indicate a decrease in the normal permeability, but merely a *decreased susceptibility to changes of permeability.* Other facts, however, such as the positive electrical variation in a muscle during inhibition and the decreased evolution of carbon dioxide, indicate, in my opinion, that there is an actual decrease in the normal resting permeability of the cell during inhibition and narcosis. The evidence from the side of electrophysiology — as

If, however, the increase of permeability is brief or momentary, the transfer of material across the plasma membrane may be too slight to produce any permanently injurious effect, an' yet sufficient to alter profoundly the metabolism of the cell. The precise effect of such a change will naturally vary from cell to cell; a muscle cell (*e. g.*) is so constructed that contraction results; in the case of the egg cell the effect — according to the present hypothesis — is the initiation of the complex mitotic process together with the associated development. The effect is obviously incommensurate with the exciting "cause"; this, however, is a distinctive peculiarity of living organisms; it is no valid objection to the present view that it is as yet impossible to account in

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interpreted on the membrane theory — is conflicting, although, on the whole, it appears to favor the present view.

The classical experiment of Claude Bernard on Mimosa furnishes conclusive proof that during ether anæsthesia there is (in this plant at least) a lessened susceptibility to influences that ordinarily increase permeability and so stimulate. Mimosæ placed in an ether-impregnated atmosphere, while retaining the normal turgor of the leaves and pulvini, lose their responsiveness to normal stimuli — *i. e.*, to influences that increase the permeability of the pulvinus cells, causing these to lose turgor and contract, with the resultant movements of the leaves. This experiment appears to me to constitute an almost unexceptionably clear proof that in anæsthesia the plasma membrane preserves its semi-permeability while losing its normal susceptibility to rapid changes of permeability. The validity of this interpretation does not seem to me to be impaired by the circumstance that Mimosa is a plant. Overton's researches have shown that the conditions of permeability are the same in plants and animals. The plant cell merely shows, in general, clearer evidence than the animal cell of the fundamental importance of changes in permeability. Bernard's experiment on the anæsthetization of the growth process in seedlings confirms the view that cell division is associated with changes of permeability. *Cf.* Claude Bernard: Leçons sur les phénomènes de la vie communs aux animaux et végétaux, Paris, 1878, J. B. Baillière, Chapter vii, pp. 259 *seq.*

detail for the ensuing transformations; the exact nature of these depends on the "inherited organization" of the cell.

I have dwelt at some length on the conditions of cell permeability in order to emphasize their critical importance for vital processes in general, as well as their bearing on the present special problem. In the latter we are now concerned particularly with the nature of the immediate effects produced by a temporary increase in permeability. The egg is to be regarded from a simple physico-chemical point of view as a chemically complex semi-fluid colloidal system enclosed by a semipermeable surface layer, the plasma membrane, which is the seat of an electrical polarization. Increase of permeability will evidently produce both chemical and physical changes in such a system: the chemical changes follow from the altered conditions of interchange with the surroundings, as already seen, and involve disturbances of chemical equilibrium in the egg; these latter, on the present theory, initiate the chemical transformations which find expression in the mitotic process. The chief physical change from the present point of view would be a decrease in the electrical potential difference normally existing between the exterior and interior of the cell (the demarcation current potential). The seat of this potential difference, on the membrane theory, is the plasma membrane, which appears to be electrically polarized in such a manner as to have its outer surface constantly at a considerably higher potential (*ca.* 0.1 volt) than its inner; this condition, the physiological polarization, is a function of the impermeability (or difficult permeability) of the plasma membrane to ions other than certain cations, — probably hydrogen ions. Hence more or less complete fall of potential, *i. e.* depolarization, must follow an increase of surface permeability sufficient to allow ready passage of anions; such depolarization (on the Lippmann-Helmholtz theorem) will be accompanied by increased surface tension. Alterations of surface tension thus induced form, in all probability, an important, if not the chief, factor in the characteristic form change of cleavage.

II. EXPERIMENTAL.

If an increase in surface permeability is in fact the primary and determinative change in cell division, there should be a relation between the ability of substances to increase permeability and their power of inciting form change or cleavage in unfertilized eggs. On the assumption that cytolysis is essentially a consequence of increased permeability, such a relation does appear to exist for many lipolytic and toxic substances, as Loeb's researches indicate. I have tested this possibility experimentally at Woods Hole during the past summer, using isotonic solutions of neutral salts of alkali metals; these solutions alter permeability — as shown by differences in the rate of exit of pigment from the eggs — with unequal rapidity. This "laking" effect is to be referred to an alteration of the colloidal consistency of the plasma membrane, since the order of relative activity of the different salts in freeing the pigment from blood corpuscles or ova agrees closely with the order of relative action on colloids.[18] The experiments about to be described also show a correspondence between the relative activity of salts in liberating pigment from unfertilized eggs, and their relative power of initiating form change or cleavage on brief exposure to the isotonic solutions.

If large quantities of Arbacia eggs are placed in test-tubes together with about twenty times their volume of pure isotonic solutions of the following salts, the red pigment is found gradually to diffuse from the eggs and to color the solutions; the effect is more rapid with sodium than with potassium salts, and the salts of each metal show a definite order of relative intensity of action. Thus, if the test tube is inverted after the eggs have remained about fifteen minutes in an isotonic sodium iodide or sodium sulphocyanate solution, the latter is found, after the eggs have settled, to be colored quite deeply by the dissolved pigment; while under the same conditions a sodium chloride solution shows little or no coloration. Later this solution also becomes colored. The order of relative action for the five sodium salts used below is: $NaCl < NaBr < NaNO_3 < NaCNS < NaI$. Potassium salts show a similar order, but act more slowly.[19] It is noteworthy that the rate of increase of permeability as thus indicated bears a direct relation to the intensity of toxic action. Thus, if eggs are transferred to sea water and fertilized with spermatozoa, after a more or less prolonged immersion in these solutions, development is always found to be interfered with or prevented, and the resulting larvæ are abnormal; if the time of exposure exceeds a certain maximum — greatest (several hours) for chloride and bromide and least (less than half an hour) for iodide — the power of development is entirely destroyed.[20] It was found that the order of relative toxicity as thus determined was essentially the same as above, and that potassium salts were less toxic than the corresponding sodium salts.

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[18] *Cf.* Höber: Biochemische Zeitschrift, 1908, xiv, p. 209. In my experiments on ova described below the usual relative order of bromide and nitrate is inverted. In their influence on colloidal aggregation state the anions usually show the order: $Cl < NO_3 < Br < CNS$ and I. Nitrate, however, showed greater action than bromide throughout the following experiments.

[19] The details of these experiments are omitted in the present paper for lack of space.

It appears thus highly probable that the injury resulting from exposure to these solutions is directly due to the abnormal increase of permeability which is produced, — and which presumably leads to a chemical disorganization of the cell through loss of diffusible protoplasmic constituents (other than the pigment).[21] Doubtless many poisons, in order to exert their characteristic effects, must penetrate into the interior of the cell; but it seems clear that a purely superficial action, by altering the normal permeability, may have equally destructive effects, as the above facts indicate. Renewed attention should be directed to the possibility that many pathological changes may result from simple alterations of the normal permeability of the cells, caused by direct action of toxines upon the constituents of the plasma membranes. Thus the hæmolytic action of many toxines is well known; Bang and Forsmann[22] have demonstrated the existence of close relations between hæmolysins and lipoids; and upon the condition of these latter substances — as the facts of anæsthesia especially indicate — the normal permeability of the plasma membrane largely depends. These facts strongly suggest that a general connection exists between abnormal increase of permeability and toxic action.

In the following experiments the eggs of both starfish (Asterias forbesii) and sea urchin (Arbacia punctulata) were used. The procedure is as follows: the eggs are collected in quantity and are allowed to settle in a mass to the bottom of the dish (finger-bowls were used of *ca.* 400 c.c. capacity). Several cubic centimetres of this mass of eggs are then transferred to a clean dry finger-bowl, the sea water is removed so far as possible, and a large volume (300–400 c.c.) of the solution to be tested is then added. When practicable, this solution is changed after the eggs have settled; if the time of exposure is brief or the specific gravity of the solution is high (as in iodide solutions), this is usually impracticable. The small quantity of sea water contained in the mass of eggs is always diluted several hundred times, and its influence is slight if not negligible. After the lapse of the experimental times of exposure the eggs are transferred by pipette to finger-bowls filled with sea water; after the eggs have settled the sea water is changed two or more times to remove any excess of the salt used in the experiment.

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[20] I am obliged to omit the details of these experiments also.

[21] The pigment is simply a convenient indicator of increased permeability; Asterias eggs which are only faintly colored are unsatisfactory for such experiments.

[22] *Cf.* Bang: Ergebnisse der Physiologie, 1909, viii, p. 477.

The solutions were made approximately isotonic with the sea water from the laboratory taps at Woods Hole. The freezing-point of this sea water, according to Garrey's determinations, is — 1.82°.[23] The molecular freezing-point depressions of most of the salts used are given in the Landolt-Börnstein-Meyerhoffer Physikalisch-chemische Tabellen, 3d edition, pages 481 *seq.*, for a somewhat small number of concentrations in most cases; the concentrations which I have used as isotonic have been obtained by interpolation from these data. In the case of sodium iodide and sodium sulphocyanate, where direct freezing-point determinations were not available, I have used solutions equimolecular with the isotonic solutions of the corresponding potassium salts (respectively, 0.53 m. and 0.55 m.); 0.53 m. NaBr was regarded as isotonic with 0.53 m. NaCl. Kahlbaum's salts were used in all of the experiments. The temperatures were always room temperatures (20° to 25°).

Experiments with starfish eggs (*Asterias forbesii*). — Comparatively few experiments were carried out with these eggs on account of the scarcity of sexually mature starfish at Woods Hole during the summer of 1909. These experiments showed conclusively that membrane formation, cleavage, and development to the blastula — or in some cases gastrula — stage could be induced in a considerable proportion of unfertilized eggs by the above treatment. Fertilization membranes are formed after very brief exposure (one to two minutes) to pure isotonic sodium chloride and similar solutions, and more readily in fully than in incompletely mature eggs; irregular form changes and cleavages, with a usually more or less abnormal development, follow membrane formation.

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[23] GARREY: Biological bulletin, 1905, viii, p. 257.

Action of isotonic solutions of various sodium salts. — The action of the four salts, NaCl, NaBr, NaI, $NaNO_3$, was compared in the series of experiments presented in Table I.

The same series was repeated next day, with exposures of five, ten, fifteen, and twenty minutes to each solution, and with essentially the same results as above. It thus appears that cell division and development may be induced by pure solutions of all four salts. Certain significant differences are also seen; iodide shows its most favorable action with brief exposures (of five to ten minutes), and produces cytolysis more rapidly than the other salts; nitrate resembles iodide rather than the other two salts in its general action; while chloride and bromide are less toxic and in general seem to give the best results with somewhat prolonged (fifteen to twenty minutes') exposure. In the above experiments the best results — as indicated by the number of eggs developing to a larval stage — were obtained after five minutes' treatment with sodium iodide. Apparently a somewhat brief and rapid action is more favorable to the initiation of division than one more gradual and prolonged. In their relative action on Arbacia eggs these salts show similar differences, as will be seen later (pp. 123 *et seq.*).

Isotonic solutions of potassium salts. — A similar series of experiments was performed with isotonic solutions of the corresponding potassium salts. The solutions used were 0.54 m. KCl, 0.51 m. KBr, 0.53 m. KI, 0.62 m. KNO_3; unfertilized mature eggs were left in each solution for respectively five, ten, fifteen, twenty, and thirty minutes and were then returned to sea water. Five minutes' exposure to 0.54 m. KCl proved insufficient to produce membranes, although a few eggs cleaved and several blastulæ were obtained; longer exposures produced membranes and a larger proportion of blastulæ. With the other salts five minutes' exposure sufficed for membrane formation and swimming larvæ were obtained in all cases, except after thirty minutes in the bromide and in the iodide solutions. Potassium salts appear to have less energetic action and to be less injurious to the eggs than sodium salts; a smaller proportion of eggs developed than in the sodium series. It was also noticeable that the potassium solutions had a distinctly less marked agglutinating action than the corresponding sodium solutions.[24]

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[24] Eggs show a tendency to cohere or "clump" after a short stay in the above solutions, especially in those of sodium salts; the order of relative action for the anions is $Cl < Br < NO_3$. Iodide, somewhat unexpectedly, showed less agglutinating action than the other salts.

TABLE I.

July 22, 1909. — The eggs from several starfish were collected, and after about three hours were transferred to the respective solutions, in which they remained for the periods indicated; they were then returned to sea water. About half of this lot of eggs failed to undergo normal maturation; the mature eggs, after exposure to the solutions and return to sea water, underwent changes as indicated; the others remained unaltered.[1]

Time of exposure to solution.	Solution.			
	A 0.53 m. NaCl.	B 0.53 m. NaBr.	C 0.53 m. NaI.	D 0.56 m. $NaNO_3$
(1) 5 minutes.	Most eggs form fertilization membranes; a fair proportion form blastulæ.	Most eggs form membranes; only a few reach the blastula stage.	Membrane formation is imperfect, but a large proportion cleave and reach larval stages (> A or B).	Most form membranes; many show good cleavage and a fair proportion form larvæ.
(2) 10 minutes.	Most form membranes; more cleavages than in A 1, and a good proportion form active blastulæ.	Most eggs form membranes; a small proportion reach blastula stage.	Deficient membrane formation; but good cleavage and a large number of larvæ.	Essentially like D 1; comparatively few larvæ.
(3) 15 minutes.	Practically the same as A 2.	Fewer membranes than in B 2; a small proportion of larvæ.	Fewer cleavages and larvæ than in C 1 or C 2.	A considerable proportion of eggs form larvæ.
(4) 20 minutes.	Membranes as above; fewer blastulæ than in A 2 or A 3.	A fair proportion form membranes and cleave; few reach blastula stage.	No membranes formed; eggs largely disintegrate; a few larvæ formed.	A few irregular cleavages; larvæ fewer than in D 3.
(5) 30 minutes.	Fewer membranes and cleavages than above; a fair number of blastulæ and some early gastrulæ.	Few membranes and cleavages; only one abnormal blastula found.	Eggs mostly break down; no larvæ.	Eggs largely break down; a small proportion form larvæ.

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[1] With the exception of those exposed for half an hour to 0.53 m. NaI, which in many instances showed irregular form changes, becoming oval or elongated with the germinal vesicle still intact but often displaced to one side of the egg. In such eggs the contents of the germinal vesicle appear perfectly water-clear and free from granules, with the exception of the nucleolus.

In the control eggs fertilized with spermatozoa there was a large proportion of irregular cleavages and the larvæ formed were largely abnormal. The unfertilized and untreated control eggs, left in sea water, were all without membranes, uncleaved, and coagulated after twenty-four hours.

A similar difference of action is seen with Arbacia eggs (see below, p. 125).

Isotonic solutions of alkali chlorides. — The action of the following four alkali chlorides was compared in one series, in which unfertilized mature eggs were exposed to solutions of 0.5 m. LiCl, 0.53 m. NaCl, 0.54 m. KCl, and 0.53 m. NH_4Cl for the same periods as above (five, ten, fifteen, twenty, thirty minutes for each salt). NH_4Cl showed little action beyond producing a few cleavages; with the other solutions results were obtained similar to those already described. Lithium and sodium chlorides showed, on the whole, a more favorable action than potassium chloride, but the differences were not sufficiently decisive to warrant any final conclusions as to relative favorability. Lack of material prevented further experiments with starfish eggs.

Experiments with sea urchin eggs. — In these experiments the following neutral salts of sodium and potassium were used: chloride, bromide, iodide, nitrate, and sulphocyanate. In their general action on unfertilized Arbacia eggs the salts were found to fall into two quite sharply contrasted groups. Chloride and bromide showed relatively slight action; while nitrate, sulphocyanate, and iodide, with favorable times of exposure, produced membrane formation, irregular changes of form, and cleavage in a large proportion of eggs. This treatment, regarded simply as a parthenogenetic method, is not very effective; only a small proportion of eggs at best developed to a larval stage in the following experiments, and the proportion undergoing irregular form change or early cleavage without proceeding far in development was always high.

Sodium salts. — The following record abbreviated from my notebook will illustrate the action of a series of four sodium salts (see Table II).

Another series with sodium chloride, bromide, iodide, and nitrate (0.56 m. $NaNO_3$) gave results essentially similar to the above. Nitrate showed a well-marked action like that of sulphocyanate and iodide, though somewhat less pronounced, while chloride and bromide showed little action as before. This distinct contrast found between bromide and nitrate — two salts which usually resemble each other in their physico-chemical and physiological action (as in their influence on colloidal aggregation state or on muscle and cilia) — was unexpected, but was confirmed in several series of experiments. In one of these

TABLE II.

August 6, 1909. — Unfertilized eggs of Arbacia were exposed for five, ten, fifteen, twenty, and thirty minutes to the following solutions: 0.53 m. NaCl, 0.53 m. NaBr, 0.53 m. NaI, 0.55 m. NaCNS. The eggs were then returned to sea water. The results were as follows:

Solution and time of exposure.	Result.
A. 0.53 m. NaCl.	
(1) 5 minutes.	No membranes formed. Practically no change.
(2) 10 “	Eggs unchanged.
(3) 15 “	Almost all eggs unchanged. A few irregular form changes.
(4) 20 “	Like A 3.
(5) 30 “	A few eggs form membranes and undergo irregular form change. Great majority unaltered.
B. 0.53 m. NaBr.	
(1) 5 minutes.	No membranes formed. No change.
(2) 10 “	Great majority unchanged. A few eggs form membranes and still fewer show form changes or irregular cleavages.
(3) 15 “	Like B 2, but somewhat more membrane formation and form change.
(4) 20 “	Like B 3. No visible change in most eggs.
(5) 30 “	A few membranes and form changes; some irregular cleavages. Most unaltered as before.
C. 0.53 m. NaI.	
(1) 5 minutes.	Marked contrast to series A and B. Membranes are separated in a large proportion; many irregular form changes and cleavages; some quite regular early cleavages. No blastulæ formed.
(2) 10 “	Similar to C_1, but more favorable: a few blastulæ formed.
(3) 15 “	Essentially like C_2; a few eggs reach blastula stage.
(4) 20 “	Less favorable. Most eggs remain unchanged after three and one-half hours in sea water; fewer membranes and form changes; no blastulæ.
(5) 30 “	Still less favorable. A good many form changes and early cleavages, but none reach larval stages.
D. 0.55 m. NaCNS.	
(1) 5 minutes.	Membranes formed in a few (< C 1); many irregular form changes and cleavages (though fewer than in C_1); two or three blastulæ found.
(2) 10 “	More favorable than D 1. A larger proportion of membranes and cleavages and a few blastulæ.
(3) 15 “	Essentially like D_2.
(4) 20 “	Essentially like D_2 and D_3. A few blastulæ formed.
(5) 30 “	Most eggs remain round and unaltered after three and one-half hours in sea water; distinctly less favorable than D_4. No blastulæ.

Controls: Unfertilized eggs left in sea water without treatment showed no change next day; eggs normally fertilized with spermatozoa formed numerous normal larvæ.

series eggs were exposed to each solution (0.53 m. NaBr and 0.55 m. $NaNO_3$) for periods of one, two, three, five, ten, fifteen, and twenty-five minutes; bromide as before produced membranes and form changes in only a few eggs and especially after the longer exposures (fifteen and twenty-five minutes), while nitrate after exposures of three minutes or longer produced a large proportion of membranes, irregular form changes and cleavages. Sodium iodide has a more rapid action than nitrate, and many eggs were found to form membranes (often imperfectly separated) and to undergo irregular form change or cleavage after exposure to the isotonic solution for less than one minute. Sulphocyanate shows an intermediate action. Bromide and chloride, although their action is slight, differ constantly from each other, the former having always the distinctly greater effect. If the relative order of action of the salts is estimated from their comparative effectiveness in producing cleavage after brief exposures (five to ten minutes), the following series is obtained: $NaCl < NaBr < NaNO_3 < NaCNS < NaI$ with a decided rise of effectiveness between bromide and nitrate. With more prolonged exposures (twenty minutes or more) iodide and sulphocyanate prove less favorable; the directly toxic action of the salt then has time to manifest itself.

Potassium salts. — In a series of experiments where unfertilized eggs were exposed to 0.54 m. KCl, 0.51 m. KBr, 0.53 m. KI, and 0.55 m. KCNS, for five, ten, fifteen, twenty, and thirty minutes in each case, results similar to those of the sodium series were obtained. Practically no effect was observed with chloride and only slight action with bromide; while with iodide and sulphocyanate the majority of eggs, with favorable times of exposure (five to ten minutes), showed form changes or irregular cleavage and a few reached the blastula stage. The development-inducing power of nitrate, sulphocyanate, and iodide was compared in a series of experiments in which an attempt was also made to determine the relative favorability of sodium and potassium salts. Eggs were exposed in one series for five, ten, twenty, thirty, forty-five, and sixty minutes to 0.53 m. NaI, 0.55 m. NaCNS, 0.53 m. KI, and 0.55 m. KCNS with results similar to those already described. Both iodides were found more toxic than the sulphocyanates and showed a briefer optimum period of exposure; the longer exposures, thirty minutes or more, were injurious or destructive to the eggs. Two additional series with NaI, NaCNS, KNO_3, and KCNS, with exposures of five, ten, fifteen, twenty, and thirty minutes, showed a similar result. The differences between the sodium and the potassium salts were indecisive. The order of relative effectiveness for brief exposures (fifteen minutes or less) was $KNO_3 < KCNS < KI$; this is also the order of increasing toxicity and increasing action in liberating pigment in isotonic solution.

Summary of above results. — In summarizing the results of the above experiments with Arbacia eggs, for both sodium and potassium salts, it is evident that the nature of the anion is the decisive factor. Potassium salts have in general a less toxic action than sodium salts upon these eggs; but in their relative power of inducing form change and development no decisive difference has appeared between the two metals. The optimum time of exposure with the three active salts is brief — in general less than fifteen minutes, and less with iodide than with the other two. With both metals a decided difference was found between chloride and bromide on the one hand, and nitrate, sulphocyanate, and iodide on the other. Bromide always has a distinctly greater effect than chloride, but this is insufficient to produce membranes and form change in more than a small proportion of eggs. The order of relative effectiveness of the anions for brief exposures is thus: $Cl < Br \mid < NO_3 < CNS < I$, the vertical line marking a critical interval in the progressive increase of action.

These facts indicate that *a relatively brief and rapid change* is more effective in starting the cleavage process than one more gradual and prolonged. The most favorable salts for initiating cell division are also the most effective in increasing permeability, as shown by the relative activity in liberating pigment from the eggs. The rapidity of the change in permeability must apparently exceed a certain critical value (above that caused by isotonic bromide solutions) in order to start the division process in these eggs.[25] It is noteworthy that Asterias eggs are more readily induced to cleave and develop by this means than Arbacia eggs, and that with the former chloride and bromide have well-marked action. This corresponds to an inherent constitutional difference between these eggs, those of Asterias being decidedly the more susceptible to development-inducing agencies, — a fact indicating on the present view that the plasma membrane of the mature Asterias egg undergoes increase of permeability more readily than that of the Arbacia egg. The latter eggs are, in fact, well known to be more resistant and hence more uniform in their behavior than those of the starfish; this difference also indicates a greater stability in the constitution of the plasma membrane of Arbacia eggs, — *i. e.*, a relatively slight susceptibility to increase of permeability.

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[25] Just as the rate of change $\left(\frac{dc}{dt}\right)$ of an electrical current traversing a muscle or nerve must exceed a critical minimum value in order to stimulate.

Experiments with alkali-earth chlorides. — Unfertilized Arbacia eggs were exposed for five, ten, fifteen, twenty, and thirty minutes to 0.35 m. solutions of magnesium, strontium, and calcium chlorides and were then returned to sea water. No result appeared beyond disintegration of the eggs; this occurred slowly after treatment with magnesium chloride, more rapidly after calcium, and still more rapidly after strontium chloride. No indication of membrane formation or cleavage was obtained. I can only suggest, as a possible explanation of the difference from alkali salts, that the initial action of the magnesium and calcium chlorides is of the opposite kind — *i. e.*, they *decrease* rather than increase the permeability of the plasma membranes.[26] Strontium chloride is strongly toxic to these eggs.

III. CONCLUSIONS.

Various more or less well-known facts, besides those already cited, support the view that the initiation of cell division is due to a critical increase in permeability, and that a rhythm of alternate increase and decrease of permeability accompanies the rhythm of the mitotic process. For the sake of completeness I shall enumerate and discuss briefly the chief of these.[27]

First, with regard to normal fertilization: that this process depends primarily on an alteration of the surface layer of the egg is suggested by its very general association with visible surface changes, such as the formation of the fertilization membrane, the separation or secretion of substances from the superficial layer of the cytoplasm,[28] and alterations of surface tension. These effects frequently appear before the sperm nucleus has undergone any evident change or penetrated far into the egg — a fact indicating that the substances which produce the change are not derived from the nucleus of the spermatozoon, but are present in its cytoplasm and possibly in its surface film. Such reasoning would point to lipolytic or similarly acting substances[29] in the surface film or plasma membrane of the fertilizing spermatozoön as responsible for membrane formation or the analogous change. Loeb and Elder have in fact recently found that membrane formation may be induced in Strongylocentrotus eggs by contact with Asterias sperm without the penetration of the sperm nucleus,[30] — a striking confirmation of the view that the primary change in fertilization is an alteration of the surface layer of the egg.[31]

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[26] The action of magnesium and calcium chlorides on Arenicola larvæ indicates a decrease of permeability, especially with the former salt. *Cf.* This journal, 1909, xxiv, pp. 25 *seq.* Magnesium salts generally exhibit an anæsthetic action.

[27] Compare the interesting short paper of E. NEWTON HARVEY on "Membrane formation and pigment migration in sea-urchin eggs," Science, 1909, N. S. xxx, pp. 694–696.

[28] The egg of the Annelid Nereis, according to my brother, Dr. F. R. LILLIE, presents a striking case of this kind. The contents of the superficial layer of cytoplasm are rapidly secreted from the egg immediately after fertilization.

Second, Lyon has found that the production of carbon dioxide in dividing eggs is not continuous, but follows a rhythm which apparently corresponds to the rhythm of cleavage.[32] He has also shown clearly that the susceptibility to injury by poisons like potassium cyanide follows a rhythm parallel to the cleavage rhythm,[33] and his results have been confirmed by Spalding[34] (using ether, acid, and potassium chloride) and by others at the Woods Hole laboratory. These experiments indicate that the entrance of poisons into the cell and the exit of carbon dioxide occur most readily at a certain period in the cycle of cell division, at or about the time of appearance of the cleavage furrow. The assumption of an increase of permeability at this time explains why the rhythms of carbon-dioxide production and of susceptibility to poisons run parallel both with each other and with the rhythm of form change (*i. e.*, of surface tension alteration).

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[29] Which might be called *membranolysins.* Loeb has called attention to the resemblance between the action of the membrane-forming substances and the chemically undefined cytolysins, Chemische Entwicklungserregung, p. 247.

[30] Loeb: *Loc. cit.*, p. 249. Professor Loeb attributes this effect to the absorption by the egg of a lysin contained in the surface layer of the spermatozöon. He believes, however, that for development the introduction of a second as yet undefined substance, possibly oxidase, into the interior of the egg is necessary.

[31] The heaping up or other disturbance of the cytoplasm at the point of entrance of the spermatozöon — a typical phenomenon in many eggs — indicates a local increase of surface tension, which would have the effect of drawing a portion of the adjoining cytoplasm toward this region.

[32] Lyon: This journal, 1904, xi, p. 52.

[33] Lyon: *Ibid.*, 1902, vii, p. 56.

[34] Spalding: Biological bulletin, 1904, vi, p. 224.

Third, if cleavage is accompanied by an increase of permeability, a negative electrical variation should accompany the formation of the cleavage furrow, just as it has long been known to accompany the stimulation process. That this is actually the case has been rendered highly probable by the experiments of Dr. Ida Hyde with the capillary electrometer, who found evidence that at the time of appearance of the cleavage furrow in Fundulus eggs the external surface of the blastodisc becomes negative relatively to the unaltered general surface of the egg.[35]

Finally, the characteristic periodic appearance and disappearance of astral formations in dividing cells are also, in my opinion, to be connected with periodic changes in permeability. Although the exact appearance presented by the mitotic figure exhibits marked variation in detail, yet the typical double system of cytoplasmic radiations centring toward two areas on either side of the altered nucleus — now in the stage of separate chromosomes — is found in the greatest variety of cells, and must depend on some condition common to them all. I have already suggested an hypothesis to account for the appearance of cytoplasmic radiations during mitosis.[36] This hypothesis is derived from the membrane theory and refers the phenomenon primarily to an alteration of the electrical surface polarization, in consequence of which a potential difference temporarily arises between the central and peripheral regions of the cell at the time of increased permeability. The arrangement of the colloidal material of the cytoplasm along radiating lines like the electrical or magnetic lines of force is due to a polarization and end-to-end juxtaposition of colloidal particles or alveoli in the electrical field thus arising. Under the conditions the fall of potential must be from without inwards, the astral centres representing regions of lowest potential or greatest negativity. I see no advantage for the present in a detailed elaboration of this hypothesis, since its essential features are clear, and it will be at once intelligible to any one conversant with the Ostwald membrane theory. The facts and considerations to be adduced in its support would thus be largely identical with those on which the membrane theory is based; and I have already in the present and preceding papers discussed the essential grounds of the present hypothesis —

[35] I. H. HYDE: This journal, 1904, xi, p. 52.

[36] R. S. LILLIE: Biological bulletin, 1909, xvii, pp. 207–208.

which are the same as those for assuming that the plasma membrane is the seat of an electrical polarization which is a function of its impermeability to ions other than hydrogen ions.[37] Increase of permeability thus entails depolarization of the membrane, and the central and peripheral regions of the protoplasm are then temporarily at different potentials. For the sake of clearness I append the following simple diagrams (Figs. 1 and 2):

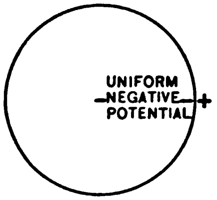

FIGURE 1. — Showing probable electrical condition of the cytoplasm during rest: a gradient of *ca.* 0.1 volt between outer and inner surfaces of plasma membrane; the potential within the cytoplasm practically uniform in all regions, and *ca.* 0.1 volt lower than the outer surface of the membrane. The cytoplasm is thus isoelectric — no gradient between its central and peripheral regions.[38]

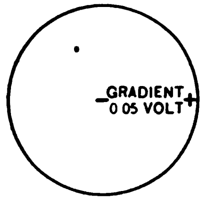

FIGURE 2. — Probable condition immediately after an increase of permeability sufficient greatly to increase the permeability of the plasma membrane to anions. The potential of the surface layer of cytoplasm becomes temporarily more positive by (*e. g.*) 0.05 volt. The cytoplasm is then no longer isoelectric, but shows a gradient of 0.05 volt between centre and periphery (equal to a gradient of *ca.* 14 volts per centimetre in a cell of the diameter of the sea urchin egg — *i. e.*, *ca.* 0.072 millimetre).

The indications that the characteristic radiations are expressions of an electrical field between the central and peripheral regions of the cell are numerous, and I have already discussed some of these — particularly the phenomena of mutual electrostatic repulsions shown between chromosomes and asters — in my earlier paper on the physiology of cell division in this journal.[39] The movements and changes of aggregation state exhibited at this time by other colloidal cell constituents support this interpretation (clearing in astral areas, migration of pigment granules, etc.). The fact that the radiations during the greater part of their existence centre toward *two* internal areas[40] seems to indicate that the increase of permeability is not uniform over the entire cell surface, but is normally greater toward the two poles; the form change is in fact best explained on the assumption of an increased surface tension in these regions;[41] corresponding to these two depolarized areas at opposite surfaces of the cell are two regions of greatest negativity in the interior.[42] The tendency of cells, particularly egg cells, to divide along a predetermined plane depends possibly on an inherent "polarity" of constitution which would also determine the properties of the plasma membrane in different parts of the surface; many biologists, as is well known, regard such polarity as a general, perhaps fundamental, characteristic of living cells. These considerations will indicate sufficiently what I believe to be the essential nature of the connection between definitely localized increase of permeability and the other more conspicuous phenomena observed in the dividing cell. The entire conditions will become clear in detail only with the advance of research, and it is best for the present to avoid a too detailed and consequently too rigid theory. That increase of permeability is a change adequate to produce the above effects seems highly probable from the foregoing considerations.

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[37] That H-ions are the penetrating cations cannot be directly proved, but many facts speak in favor of this assumption. *Cf.* This journal, 1908, xxii, pp. 86, 87.

[38] Probably the nuclear membrane is also the seat of a potential difference, but there is no very direct evidence of this as yet. In these diagrams a possible influence of the nucleus is provisionally disregarded.

[39] R. S. LILLIE: This journal, 1905, xv, p. 46.

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[40] I am not considering here the case of polyastral formations which appear frequently in eggs treated by the methods of artificial parthenogenesis. *Cf.* E. B. WILSON: Archiv für Entwicklungsmechanik, 1901, xii, p. 531.

[41] ROBERTSON: Archiv für Entwicklungsmechanik, 1909, xxvii, p. 29.

[42] This hypothesis, however, does not account for the characteristic arrangement of the radiations with reference to the nucleus, — a condition indicating that the nuclear or interastral area in the normal mitosis has a higher potential than the astral centres, *i. e.*, is positive relatively to these. There are various reasons for assuming this. It appears quite inadmissible to suppose the two astral centres to be oppositely charged: such a condition would involve a contrast in physico-chemical behavior which does not exist. Yet the course of the radiations between adjacent centres shows the spindle form characteristic of the lines of force between opposite electric or magnetic poles: this fact is inconsistent with the assumption of similarity of charge if the asters alone are held responsible for the observed configuration. These difficulties partly disappear on the assumption that the nuclear region has permanently a higher potential than the cytoplasmic region, *i. e.*, that it fails for some reason to assume the uniform negative potential ascribed above to the rest of the protoplasm, and hence remains positive relatively to the latter — hence relatively to the astral centres at the time of mitosis. But how to account for such a condition? Possibly the existence of a semi-permeable membrane between nuclear and cytoplasmic regions, impermeable to ions, *except during mitosis*, is the essential factor. Such a membrane would prevent the area which it enclosed from assuming the negative potential of the rest of the protoplasm. The nuclear membrane would thus mark the boundary of a region with a higher potential than the rest of the cell inside the plasma membrane. Whatever the conditions, it seems at least clear that the nucleus is responsible for the altered course of the astral radiations in its vicinity. — The nuclear membrane, like the plasma membrane, is in all likelihood the seat of a potential difference, and changes in its permeability would produce analogous effects. Asters — the signs of local potential differences — do in fact frequently appear first near the regions where the nuclear membrane begins its dissolution in the prophase of mitosis.

The nature of the chemical changes induced in the egg by the parthenogenetic treatment is now in its earliest stages of investigation. Free oxygen is necessary to the progress of cell division in most cases; and in some eggs — as Loeb has shown for Strongylocentrotus — although oxygen is not necessary for membrane formation its presence appears to be required during the treatment with hypertonic sea water, if favorable development is to result. On the other hand, in Asterias parthenogenesis is best produced if the eggs are subjected to the required treatment under conditions that suppress or inhibit oxidations, according to Delage's[43] and my own[44] results (absence of oxygen or presence of potassium cyanide). Such differences between different eggs cannot be explained at present. On the above theory they would indicate differences in the physico-chemical constitution of the plasma membrane; the latter must be regarded as itself complexly organized and the seat of a metabolism which differs in different forms; and conceivably the critical changes of permeability may in some cases be favored by an increase, in others by a decrease, in the rate of oxidations. As I pointed out in my former paper,[45] the initiatory process may require quite different conditions from those needed for the continuance of development once this is begun. In Asterias eggs, so far as the present evidence indicates, temporary suppression of oxidations seems favorable to the artificial initiation of normal development; in sea-urchin eggs the reverse appears to be true. Both eggs alike fail to develop in the absence of oxygen.[46]

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[43] DELAGE: Comptes rendus, 1907, cxlv, p. 218.

[44] R. S. LILLIE: Journal of experimental zoölogy, 1908, v, p. 375.

[45] *Loc. cit.*, p. 427.

[46] Professor LOEB has explained the apparent discrepancy between his own re-

IV. SUMMARY.

The chief facts and general conclusions of the foregoing paper may be briefly summarized as follows:

1. Unfertilized eggs of Arbacia placed in pure solutions of the various neutral salts of the alkali metals isotonic with sea water, show, after varying intervals, diffusion of pigment into the medium and eventually disintegration. The salts of the same metal differ in their rate of action; the order of relative effectiveness for sodium salts with monovalent anions is: $NaCl < NaBr < NaNO_3 < NaCNS < NaI$. Potassium salts have a more gradual effect of the same kind and show the same general order of relative activity. The loss of pigment is an effect analogous to hæmolysis and indicates an increase in the permeability of the plasma membrane, due probably to change in the aggregation state of its colloids.

2. If eggs, after a relatively brief exposure (five to twenty minutes) to these solutions, are transferred to normal sea water, a certain pro-

sults with Strongylocentrotus, and DELAGE's and mine with Asterias, as due to a misunderstanding, on both our parts, of the nature of his results. Exposure of Strongylocentrotus eggs, for some time subsequently to artificial membrane formation, to sea water containing cyanide or deprived of oxygen, always increases the proportion of favorably developing eggs; our results simply show the same to be true of starfish eggs; there is thus no real discrepancy. Treatment with cyanide after membrane formation by momentary warming does indeed increase the proportion of favorably developing starfish eggs (*cf.* my paper, *loc. cit.*, pp. 424–425). But it is equally true, and in my opinion equally significant, that exposure to cyanide for some time *before* membrane formation — which is followed by *immediate* return to normal sea water — also gives results far superior to those following simple warming without cyanide treatment (*Loc. cit.*, pp. 415–419). Here there can be no question of suppression of development after membrane formation, so that the egg protoplasm may have time to return to a condition favorable for development — unless indeed the improvement is attributed to the persistence of the cyanide effect after the return to normal sea water. But mere after treatment with cyanide is relatively ineffective. The fact is that in Asterias (as I was careful to point out) the *whole* procedure of the initiation of development by brief warming is best carried out in the presence of cyanide. The best results were gained when eggs in the early maturation stage were exposed for about an hour to sea water containing *m*/2000 KCN, then warmed briefly to 35° in the same medium, and then returned for a brief period (five to twenty minutes) to the cyanide solution; thence to normal sea water. With this procedure the optimum exposure to cyanide *before* warming is several times longer than the after exposure.

portion — small after treatment with chloride or bromide, but considerable or large after nitrate, sulphocyanate, and iodide — exhibit membrane formation, irregular form change, and cleavage, and a small proportion may develop to the blastula stage. The order of relative favorability for the different salts is the same as above. This agreement confirms the view that the primary change in the initiation of cell division and development is an increase in the permeability of the plasma membrane.

3. The above order is also that of relative toxicity. The toxic effect is to be attributed mainly to the loss of diffusible cell constituents through the altered plasma membrane; the exit of pigment is a visible instance of this kind. The irreversibility of the death process — as contrasted (*e. g.*) with the reversibility of anæsthesia where the normal permeability appears to be *decreased* rather than increased — is a correlate of the irreversible chemical disorganization which results from extensive loss of the diffusible protoplasmic constituents through the now permeable plasma membrane.

4. The initiation of cleavage is similarly to be referred to altered conditions of interchange of diffusible substances and ions across the plasma membrane during the initial period of temporarily increased permeability. Disturbances of chemical equilibrium thus result which alter the character of cell metabolism in such a way as to start the developmental processes. The changes in the form of the cell and in the configuration of the cytoplasm (astral radiations) during mitosis are referred directly to altered electrical polarization of the plasma membrane consequent on increased ionic permeability. A rhythm of alternate increase and decrease of permeability thus accompanies the rhythm of the mitotic process.

THE METABOLISM OF DEVELOPMENT.—I. ENERGY METABOLISM IN THE PREGNANT DOG.

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INTRODUCTION.

METABOLISM, as the term is now generally understood, includes the chemical changes by which the living substance is produced as well as those by which it is maintained. The term also includes the chemical transformations by which energy, for the various purposes to which it is put in the organism, is made available, whether in the developing cell or in the mature cell. Thus the terms substance metabolism and energy metabolism, which are now coming into general usage as the equivalents of the German Stoffwechsel and Kraftwechsel must be conceived as applying to the developing organism as well as to the mature organism.

The two sets of processes denoted by these terms are by no means entirely independent of each other, although they probably rest upon fundamental properties of the living substance which are quite distinct.[1] These properties, as nearly as they can be denominated at present, are, on the one hand, the ability to attract and impress into its own structure germane materials, and, on the other, the ability to liberate, in most cases by oxidation, the energy supplied to it in potential form. Because one is a synthetic tendency and the other an analytic tendency, these two properties have frequently been regarded as direct opposites, and the terms anabolism and katabolism, introduced by English physiologists, have served to emphasize this distinction. But these latter terms have led to some confusion. Introduced at a time when Liebig's view of the coincidence of substance and energy changes was the prevailing view, they have served through indiscriminate usage to prolong the life of an idea long past its usefulness. Liebig believed that whenever new protoplasm is formed energy is stored and that the liberation of energy necessarily involves a destruction of protoplasm. But from our present knowledge of the constitution of the protein substrate of the living protoplasm, it requires but a moment's reflection to convince one that the conversion of, say, meat protein of the ox to tissue protoplasm of a man or dog, assuming that no reduction in the chemical sense is necessary to vitalize this protein, involves no storage of energy,[2] and it has been abundantly proven by the Voit school of investigators that the destruction of the living substance (as measured by the output of nitrogen) is by no means proportional to its activity, *i. e.*, to the amount of energy liberated.[3] Hence the antithesis of constant building up and tearing down of the living substance as a condition of energy release, which the terms anabolism and katabolism have mistakenly been used to emphasize, can no longer be regarded as true. All living substance apparently suffers continually a certain small loss of nitrogen, but this loss is not of necessity a condition of energy release, especially if the separation takes place by hydrolysis.[4] It should then be recognized that processes of anabolism (building up of materials under the influence of the living substance) do not of necessity involve a storage of potential energy, and that processes of katabolism (breaking down of materials under the influence of the living substance) may take place without release of any energy.

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[1] RUBNER: "Man soll also die energetische Leistungen von den Stoffwechselsveranderungen scharf scheiden," Archiv für Hygiene, 1908, lxvi, p. 32; also "Die Mehrung der lebenden Substanz hat mit dem Kraftwechsel selbst nichts zu tun d. h. beides sind getrennte und wohl zu scheidende Funktionen," *Ibid.*, p. 35.

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[2] This has been made practically certain by the calorimetric studies on the heat of combustion of proteolytic digests before and after digestion, made by LENGYEL (Archiv für die gesammte Physiologie, 1906, cxv, p. 7) and by HARI (*Ibid.*, p. 11); and the more recent attempt made by GRAFE (Archiv für Hygiene, 1907, lxii, p. 216) to detect a heat production in proteolysis by means of an extremely sensitive calorimeter. Both methods show that no heat whatever is generated by cleavage of proteins. If our assumption that synthesis of proteins from these same cleavage products is the essential thing in the formation of new protoplasms is true, then their construction can be accompanied by no production of energy in the sense in which this occurs when starch is formed from CO_2 and H_2O.

[3] See, *e. g.*, VOIT, Zeitschrift für Biologie, 1866, ii, p. 339; FICK: Vierteljahrschrift der Zürischer naturforscher Gesellschaft, 1865, x; KRUMMACHER: Zeitschrift für Biologie, 1896, xxxiii, p. 108; ATWATER *et al.*: United States Department of Agriculture, Office of Experiment Stations, Bulletins Nos. 89 (1901) and 117 (1902); FRENZEL, Archiv für die gesammte Physiologie, 1897, lxviii, p. 212; and many others. SHAFFER: This journal, 1908, xxii, p. 445, has recently shown that there is not only no increase in the total nitrogen output in muscular work, but that the various nitrogen fractions have the same relation to each other as in muscular rest.

[4] See ZUNTZ: Centralblatt für Physiologie, 1908, xxii, p. 67.

The living substance, as a matter of fact, so long as it is living and under the conditions usually termed normal, seems to be relatively stable, so far as oxidation is concerned, but it is endowed with the property of "activating" oxygen, and this enables it to bring about the oxidation of inert materials (fats, carbohydrates, and, after splitting off of the nitrogen, of nitrogenous materials as well) which may be brought into an intimate enough relationship with it. The growth or maintenance tendency of the living substance certainly is not the opposite of this, but is probably to be looked upon as a sort of polymerization whereby unitary components of the living substance attract similar unitary chemical structures which cannot themselves be described as living, but which take on the vital character in virtue of their incorporation into the more complex relationship. That the process of oxidation is a condition essential to the continuance of this polymerization is, perhaps, a safe inference from its almost universal association therewith.[5]

Since the chemical basis of the living substance is essentially protein in nature, the study of the substance metabolism resolves itself mainly into a study of the chemical changes in the protein materials entering the organism as food, from the moment their digestion begins until their reappearance in the external world as waste. The study of the energy metabolism, on the other hand, concerns itself mainly with the oxidation of non-nitrogenous materials.[6] One is obliged to say "mainly" in both cases, because, in the first place, certain cells always normally, and most cells at times, contain non-nitrogenous materials which fluctuate in amount with the nutritive condition of the living substance, and in this sense are to be regarded as a part of it, and, in the second place, some energy probably always is derived from what were, before deamidation, nitrogenous materials.

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[5] See p. 138.

[6] *Cf.* RUBNER: "Beim Eiweiss drängt sich in der Ernährung die substantielle Frage, beim Fett und Kohlehydrat die dynamogene in den Vordergrund," *Loc. cit.*, p. 34.

DIFFERENCES IN METABOLISM BETWEEN DEVELOPING AND ADULT ORGANISMS.

It is already clear that the chemical processes by which the living substance is maintained are not identical with those by which it is originally produced. We know, for example, that growth and division of the nuclei are essential in the production of new tissues, while the mere replenishment of cell materials, such as is taking place continually on a small scale or such as may take place in convalescence on a large scale, may go on without division of the nucleus. Since it is known that the nucleus is essential to processes of intracellular digestion,[7] it is possible that the nucleus plays some essential rôle in this process of replenishment; but the fact that the nucleus itself does not grow and divide under these circumstances,[8] together with the fact that its reactions and constitution are known to be different from those of the cytoplasm,[9] makes it very probable that growth involves chemical processes not concerned in the replenishment which follows ordinary waste or that which follows extraordinary waste in diseased conditions. Rubner[10] has drawn attention to the fact that the maintenance tendency is of primary importance even in the young organism, since the "wear and tear" quota (Abnutzungsquote) must be satisfied before growth (postembryonic) of the organism as a whole can assert itself. If we assume that the every-day repair concerns mainly the cytoplasm, except where cells are actually being destroyed, Rubner's view might be interpreted to mean that the processes in the nucleus which result in its growth and division can take place, even in the young organism, only under certain optimum nutritive conditions of the cytoplasm. It is my intention to deal with some phases of the substance metabolism in development more fully in a subsequent paper. At present the possible differences between the developing and the adult organism are mentioned only for the purpose of introducing the possible differences in the energy metabolism.

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[7] *Cf., e. g.*, VERWORN, Zeitschrift für wissenchaftliche Zoölogie, 1888, xlvi, p. 455, and Archiv für die gesammte Physiologie, 1892, li, p. 1; HOFER, Jenaischer Zeitschrift für Naturwissenschaft, 1889, xvii, p. 105; MISS GREENWOOD, Journal of physiology, several articles, 1886–1890.

[8] *Cf.* LOEB: Archiv für die gesammte Physiologie, 1894, lvi, p. 270; LOEWENTHAL: Zeitschrift für Nervenheilkunde, 1898, xiii, p. 106; VIRCHOW: Cellularpathologie, 4th ed., 1871, p. 365; and ADAMI, Principles of pathology, 1908, i, p. 541.

[9] MIESCHER, A. B. MACALLUM, KOSSEL, A. P. MATHEWS.

[10] *Loc. cit.*, p. 81.

There is no reason for thinking that the mechanism by which energy is liberated in young cells is different from that which performs the same service in mature cells. The living substance of all cells (with the exception of the anærobic forms) is dependent upon some power, call it the "activation of oxygen," whereby oxygen becomes capable of uniting with the elements of the soluble food-stuffs at a temperature much below the ordinary kindling temperature. We need not delay to discuss the different theories which have been advanced to account for this "activation." It has been rendered very probable, by the observations of Spitzer [11] and R. S. Lillie,[12] that the nucleus plays an essential part in this function in resting cells, and Loeb,[13] on the evidence obtained by him and by others, suggests that the reason why an enucleated cell cannot grow is because the synthetic processes are dependent upon the oxidative changes brought about through the nucleus.

Warburg's [14] recent observation that fertilized sea urchin eggs absorb six to seven times as much oxygen in the same length of time as do unfertilized eggs, lends weight to the view that oxygen is in some way essential to the growth process, but his further observation that there was no proportion between the amount of oxygen absorbed and the number of nuclei (blastomeres) present, and that still more oxygen was absorbed when the eggs were placed in hypertonic solutions and cell divisions had ceased,[15] certainly do not favor the idea that oxygen absorption is dependent on nuclear activity. This is in accordance with Rubner's [16] view that the morphological changes in the nucleus accompanying cell division are the expression of synthetic processes rather than of the destructive processes of oxidation. Lyon's [17] suggestion (based upon an observed rhythmical susceptibility to lack of oxygen and a possible rhythmical production of carbon dioxide by segmenting sea urchin eggs) that the oxygen is used chiefly for syntheses during the growth phase of cell division, while the carbon dioxide is liberated chiefly at a time when the cytoplasmic mass is in actual division, may serve eventually to harmonize these observations; but the facts are as yet insufficient to warrant such a conclusion.

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[11] Spitzer: Archiv für die gesammte Physiologie, 1895, lx, p. 303.

[12] Lillie: This journal, 1902, vii, p. 412.

[13] Loeb: Archiv für Entwickelungsmechanik, 1899, viii, p. 689.

[14] Warburg: Zeitschrift für physiologische Chemie, 1908, lvii, p. 1.

[15] Warburg: *Ibid.*, 1909, lx, p. 443.

[16] Rubner: Archiv für Hygiene, 1908, lxvi, p. 146.

[17] Lyon: This journal, 1904, xi, p. 52.

Whatever be the explanation from the standpoint of the individual cell, however, there is no doubt that embryonic development does occasion a more active production of carbon dioxide per unit of mass than takes place in adult tissues. This has been demonstrated by Farkas[18] for the eggs of the silkworm, by Bohr[19] for the embryo snake, by Bohr and Hasselbach, and by Hasselbach alone[20] for the developing chick, and by Bohr[21] for the embryo guinea pig. That this greater evolution of carbon dioxide is the expression of a greater liberation of energy also is rendered perfectly certain by the calorimetric measurements made by Farkas[22] of the heat of combustion of unincubated and incubated silkworm eggs, and those of Tangl[23] on the eggs of the cadaver fly; by similar measurements made by Tangl[24] and by Tangl and Mituch[25] on unincubated and incubated hen's eggs; and by the direct calorimetric determination of the heat produced in the developing hen's egg made by Bohr and Hasselbach.[26]

Specifically, Bohr and Hasselbach found on the fifth day of incubation of the hen's egg a production of CO_2 amounting to 2000 c.c. per kilogram of embryo per hour as against 718 c.c. per kilogram and hour for the adult hen (Regnault and Rieset). The CO_2 production from the eighth to the twenty-first day (end) of incubation was only a little greater in the embryo than in the adult hen, but was sufficiently high for the authors to feel justified in concluding that it was "a condition for the organization of the new tissue and not alone for the maintenance of tissues already formed.[27] Grafe,[28] in reviewing this work, lays special emphasis on the fact that the highest energy production takes place at a time when the work of differentiation is most active. Bohr had previously supported this view with the evidence derived from his study of embryo snakes.[29] Increasing the temperature from 15° C. to 27° C. increased the CO_2 output of an embryo weighing 3.8 gm. about 2.8 times, while the same increase in temperature raised the output of an embryo weighing 0.5 gm. exactly four times. The greater increase produced in the younger embryo, Bohr believes, was due to the greater change in the intensity of the developmental processes. That is, the processes of new formation (differentiation) are more active in the younger stage and it is this part of the developmental process which is responsible for the more active metabolism.

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[18] FARKAS: Archiv für die gesammte Physiologie, 1903, xcviii, p. 490.

[19] BOHR: Skandinavisches Archiv für Physiologie, 1904, xv, p. 23.

[20] BOHR and HASSELBACH: *Ibid.*, 1900, x, pp. 149, 353.

[21] BOHR: *Ibid.*, 1900, x, p. 413.

[22] FARKAS: *Loc. cit.*

[23] TANGL: Archiv fur die gesammte Physiologie, 1909, cxxx, p. 1.

[24] TANGL: *Ibid.*, 1903, xciii, p. 327.

[25] TANGL and MITUCH: *Ibid.*, 1908, cxi, p. 437.

[26] BOHR and HASSELBACH: Skandinavisches Archiv für Physiologie, 1903, xv, p. 398. See also WEINLAND'S paper on the metamorphosis of the blow-fly, Zeitschrift für Biologie, 1906, xlvii, p. 186; that of TANGL and FARKAS on the eggs of the trout, Archiv für die gesammte Physiologie, 1904, civ, p. 624; and that of GODLEWSKI, on frog's eggs, Archiv für Entwickelungsmechanik, 1901, xi, p. 585. All of these observers measured the rate of metabolism, but did not compare the embryonic directly with the adult metabolism.

Tangl's results on the hen's egg indicate an average heat production for the entire incubation period of 100 calories per kilogram per day as against 71 calories per kilogram per day (at 18°–20°) for the adult hen found by E. Voit [30] — an increase of 41.3 per cent. Tangl concludes that the energy required for development (Entwickelungsarbeit) is considerably greater than that required for mere maintenance of the adult organism (Erhaltungsarbeit). The difference he designates as Bildungsarbeit.[31] Bohr's findings on the pregnant guinea pig [32] are not so convincing. The average production of CO_2 in the embryo [33] he found to be 509 c.c. per kilogram and hour; that of the mother 462 c.c. per kilogram and hour — an increase of only 10 per cent. Granted that the conditions of heat loss were the same in the two, which is doubtful, the amount of metabolism which could be ascribed to any developmental process as opposed to the maintenance processes would be very small.

[27] BOHR and HASSELBACH: *Loc. cit.*, x, p. 149.

[28] GRAFE: Biochemisches Centralblatt, 1907, vi, p. 441.

[29] BOHR: Skandinavisches Archiv für Physiologie, 1904, xv, p. 23.

[30] E. VOIT: Zeitschrift für Biologie, 1901, xli, p. 120.

[31] TANGL: Archiv für die gesammte Physiologie, xciii, p. 362.

[32] BOHR: Skandinavisches Archiv für Physiologie, 1900, xv, p. 413.

[33] Estimated by noting the difference in the output of the mother, under anæsthesia and immersed in a warm bath, when the umbilicus of a single embryo was clamped off.

Rubner[34] believes that his dynamic law of skin area[35] is applicable to the embryo. He calculates that the metabolism of the new-born mammal, assuming its weight to be 8 per cent of that of its mother, would be nearly double as much per kilogram and hour as that of the mother. Because the embryo is less active in every way than the new-born, its metabolism per unit of weight should be considerably less than this, which indeed the results of Bohr and of Tangl show to be the case. Rubner explains the higher metabolism of the embryo per unit of weight, therefore, as due not to any specific requirement for developmental energy, but entirely to the greater loss of heat by the relatively greater surface. He is obliged, however, to eliminate the first four tenths[36] of the embryonic life from the operation of this law, because within that period the embryo is of no appreciable size as compared with the mother. On the basis of the average composition of living substance in mammals and using seven tenths of the metabolism of the new-born as the rate for the embryo, Rubner calculates that for the remaining six tenths of the gestation period the "growth quota" of the embryo in most mammals is from 38 to 40 per cent of the energy supplied, as contrasted with 34 per cent for extra-uterine life. In other words, for each calorie of heat value stored in the new-born, nearly two calories of energy must be expended, while for each calorie deposited in the embryo[37] only one and one-half calories need be expended (on the basis of 40 per cent).

QUALITATIVE DIFFERENCES.

The qualitative differences in the metabolism of the embryo from that of the adult depend on the kind of food material supplied by the mother in the egg (oviparous development) or by the circulation (viviparous) for the nutrition of the embryo. A hen's egg contains no carbohydrate; hence the respiratory quotient in development of the chick can never be greater than 0.78. The chemical studies of Liebermann,[38] the calorimetric determinations of the heat of combustion by Tangl,[39] and the metabolism studies (using both the direct and the indirect methods) by Bohr and Hasselbach[40] all agree in showing that the material oxidized in the development of the chick is fat. Liebermann believed that some nitrogen was lost, but both Hasselbach[41] and Tangl and Mituch have shown that this is incorrect. The nitrogenous building material is not used as a source of energy.

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[34] RUBNER: *Loc. cit.*, p. 180.

[35] See LUSK: Elements of the science of nutrition, 2d edition, p. 89, Philadelphia, 1909, for an elucidation of this law.

[36] This calculation is based on the guinea pig and man.

[37] TANGL had previously shown (*Loc. cit.*, 1903, xciii, p. 327) that the amount used in the complete development of the chick is only one calorie for each two calories laid down.

[38] LIEBERMANN: Archiv für die gesammte Physiologie, 1888, xviii, p. 71.

The source of energy for the silkworm embryo, according to the chemical studies of Tichomiroff[42] and the respiration experiments of Farkas;[43] for the blow-fly embryo according to the respiration experiments of Weinland;[44] and for the cadaver fly according to the calorimetric determination of Tangl[45] is likewise mainly fat. No nitrogen is lost in gaseous form during the development of any of these insects, but a portion of the energy (according to Farkas approximately one third) arises from the oxidation of proteins to uric acid.[46] Both Tichomiroff for the silkworm egg and Weinland for the blow-fly recorded a reduction of the glycogen content of the egg, but Weinland believes this may have been converted to chitin. There is no evidence, he says, that glycogen has served as a source of energy.

Our information as to what material is the source of energy for the mammalian embryo is extremely scanty. Cohnstein and Zuntz[47] analyzed the blood in the umbilical artery and vein of the sheep embryo for oxygen and carbon dioxide, and noted a difference of 4.67 vols. per cent O_2 and 4.72 vols. per cent CO_2 in one case and 4.0 vols. per cent O_2 and 6.5 vols. per cent CO_2 in another. These figures would give respiratory quotients of 1.01 and 1.6, respectively, for the two embryos. It is doubtful whether these figures are to be trusted, since on the basis of the same analyses the authors claim a metabolism for the embryo of only one fourth to one sixth as much per unit of weight as for the mother. The quotients agree, however, with those found by Bohr[48] on the embryo of the guinea pig. Bohr took the difference between the total gaseous exchange of the pregnant animal (after operation under anæsthesia and immersed in a warm bath) before and after clamping off a single umbilicus. The respiratory quotient indicated for the embryo was always in the neighborhood of unity. Oddi and Vicarelli[49] report also a progressive increase in the respiratory quotient in the course of pregnancy in the mouse. According to these observations, therefore, the most diffusible of the foodstuffs, the one most readily passed through the placenta,[50] is probably the source of energy for the mammalian embryo. There is no satisfactory evidence as yet that proteins participate to any considerable extent in furnishing such energy.

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[39] Tangl: *Loc. cit.*, 1903.

[40] Bohr and Hasselbach: *Loc. cit.* and *Ibid.*, 1903, xv, p. 398.

[41] Hasselbach: *Loc. cit.*, 1900.

[42] Tichomiroff: Zeitschrift für physiologische Chemie, 1885, ix, p. 518.

[43] Farkas: Archiv für die gesammte Physiologie, 1903, xcviii, p. 490.

[44] Weinland: *Loc. cit.*

[45] Tangl: *Loc. cit.*

[46] Tangl and Farkas observed no change in the N-content of developing trout eggs.

[47] Cohnstein and Zuntz: Archiv für die gesammte Physiologie, 1884, xxiv, pp. 230, 231.

To summarize the evidence to date, then, we find, in general, (1) that the energy metabolism of the embryo is greater per unit of weight than that of the mother, and (2) that this energy is derived in oviparous animals almost entirely from fat and in viviparous animals almost exclusively from carbohydrate.

METABOLISM IN PREGNANCY.

In the light of these facts what change should we expect in the energy metabolism of the pregnant organism as contrasted with the same organism in sexual rest? First, it is clear that the energy requirement of the embryo alone should be progressively greater, so far as absolute measurements are concerned, as the mass of embryonic tissues increases. Whether this increase is proportional to the increase in weight or to the increase in superficial area of the embryo, as Rubner believes to be the case for the latter six tenths of the gestation period at least, has not yet been determined by actual observations. In the second place, the energy requirement of the mother should likewise be progressively increased by the demands of the accessory maternal structures — uterus, placenta, mammæ, etc. The fœtal fluids (amniotic and allantoic), on the other hand, should tend of themselves to diminish the total metabolism of the pregnant organism per unit of weight.

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[48] BOHR: *Loc. cit.*, 1900.

[49] ODDI and VICARELLI: Archives italiennes de biologie, 1891, xv, p. 367, reviewed in Centralblatt für Physiologie, 1891, v, p. 602.

[50] The passage of dextrose through the placenta has been definitely proved by COHNSTEIN and ZUNTZ, Archiv für die gesammte Physiologie, xlii, p. 342, by LUDWIG, Zentralblatt für Gynäkologie, 1895, xix, p. 281, and others. See L. ZUNTZ: "Der Stoffaustauch zwischen Mutter und Frucht," in Die Ergebnisse der Physiologie, 1908, vii, p. 430. See also recent literature on glycogen in embryonic tissues, *e. g.*, MENDEL and LEAVENWORTH, This journal, 1907, xx, p. 117.

But few experiments have been made to determine the relative rate of the metabolism at different stages of the pregnancy, and the few that have been made are confined to the respiratory metabolism. Andral and Gavarret[51] made determinations of the CO_2 output in several pregnant women and noted a progressive increase as follows: third month, 7.8 gm. C. per hour; fifth month, 8.1 gm. C.; eighth and one half month, 8.4 gm. These figures were not, however, obtained on the same subject; hence are of little value. Reprew,[52] using the apparatus of Paschutin in St. Petersburg, found in three experiments on rabbits, one on a guinea pig, and one on a dog that the absorption of oxygen decreased as pregnancy advanced, but the elimination of carbon dioxide fell still more, so that there was a progressive decline of the respiratory quotient. Oddi and Vicarelli,[53] working with the apparatus of Luciani and Picetti modified by Oddi, found exactly the reverse relationship in white mice. Both the oxygen absorption and the carbon dioxide elimination increased, but the latter to a greater extent than the former, so that the respiratory quotient increased. The only experiments on women yet reported have been done with the Zuntz apparatus, and only one such experiment has been published in any detail. This experiment, done by Magnus-Levy in 1896 and 1897 was reported by him in an address before the Gesellschaft für Geburtshülfe und Gynäkologie in Berlin in 1904.[54]

His subject, a woman of large build, weighing in the non-pregnant condition 108 kg., showed a progressive increase in the absorption of oxygen from 302 c.c. per minute in the non-pregnant condition to 383 c.c. per minute in the ninth month. Reckoned per kilogram of body weight, there was also a slight increase as follows:

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[51] ANDRAL and GAVARRET: Annales de chemie et de physique, 1843, 3° ser., viii, p. 129.

[52] REPREW: According to a translation of the original made for me by Dr. A. I. RINGER, REPREW's results are due to differences in temperature; also Ueber den Einfluss der Schwangerschaft auf den Stoffwechsel bei Thieren, Innaug.-Dissert. St. Petersburg, 1889, mentioned by, ZACHARJEWSKI, Zeitschrift für Biologie, 1894, xxx, p. 368.

[53] ODDI and VICARELLI: *Loc. cit.*

[54] MAGNUS-LEVY: Zeitschrift für Geburtshülfe und Gynäkologie, 1904, lii, p. 116.

Non-pregnant	2.79 c.c. O_2 per kilo per min.
Third month	2.88 c.c. O_2 per kilo per min.
Fourth month	2.92 c.c. O_2 per kilo per min.
Fifth month	3.16 c.c. O_2 per kilo per min.
Sixth month	3.14 c.c. O_2 per kilo per min.
Seventh month	3.10 c.c. O_2 per kilo per min.
Eighth month	3.20 c.c. O_2 per kilo per min.
Ninth month	3.33 c.c. O_2 per kilo per min.

In discussing Magnus-Levy's paper L. Zuntz reported at the same meeting other experiments performed by Franz Müller in which no increase of the oxygen absorption per kilogram could be demonstrated up to the fifth month, and L. Zuntz, in his Ergebnisse article,[55] mentions two cases in which he himself could find no clear evidence of an increased absorption.

ORIGINAL OBSERVATIONS.

With the idea of testing Magnus-Levy's results the writer undertook a couple of years ago some experiments on the total metabolism of the pregnant dog, using the smaller Voit-Pettenkoffer apparatus for the determination of the carbon dioxide output. Because of the difficulty of maintaining a constant temperature in the respiration chamber as usually constructed for this apparatus and the impossibility of determining the oxygen absorption with any satisfactory degree of accuracy, only one experiment covering the entire gestation period was completed. Toward the end of this period, however, a constant-temperature cage was constructed and by the help of this the writer was able to secure a very satisfactory comparison of the total metabolism of the mother and a single puppy just before and just after birth with that of the mother dog alone in sexual rest two weeks after lactation had ceased. This was repeated six months later on the same dog in a second pregnancy from which five puppies were born.

[55] ZUNTZ: *Loc. cit.*

PROTOCOL.

First pregnancy. — The dog, a female bull terrier, weighing 12.3 kg. on April 21, was copulated by a male of the same breed on April 22, 1908. A single puppy was born on June 26. The dog was kept in the respiration chamber on two days of each week of the gestation period and, together with the puppy, on the first day following parturition. The dog was on a measured diet every day of the gestation period and for several weeks after parturition. The urine was collected daily for the same period. On days of the respiration experiments the daily routine began about five P. M. The dog was catheterized, then was weighed and fed, and the feces were obtained on a platter. Immediately thereafter the dog was placed in the chamber and kept for twenty-two hours, allowance being made for a short halt in the morning for the purpose of drawing the urine, by running as much later in the afternoon. What remained of the twenty-four hours was employed in exchanging the barium hydrate tubes, re-standardizing the meters and attending to the food, urine, etc. The food, with the exception of the first week, contained approximately the same amount of nitrogen and potential energy throughout, and from the beginning of the fifth week to the end of the experiments contained exactly the same amounts. This daily diet follows:

275 gm. beefheart (3 % N and 5 % fat)	8.250 gm. N and	339.2 cal.
40 gm. lard		372.0 cal.
50 gm. crackermeal (1.48 % N)	0.740 gm. N and	196.2 cal.
10 gm. bone ash	0.0	0.0
2 gm. salt	0.0	0.0
Total	8.990 gm. N and	907.4 cal.

Sexual rest. — The puppy was taken away from the mother at the end of a week, but the mother dog was kept in the same cage and on the same diet until July 15. After spending the summer in the country the dog was brought into the laboratory again in September and was placed on a measured diet early in October.

Second pregnancy. — Signs of menstruation were observed on October 4 and on the 10th the male was taken. Copulation was permitted again on the 11th and a third time on the 12th. The urine was collected throughout this pregnancy for the purpose of following the protein metabolism, and for this purpose the diet was varied somewhat, but at the time the respiration experiments were begun (December 10) the dog had been on the old diet for two days.

The results are given in brief in Tables I and II, in more detail in Table III, at the end of the paper. The total energy production is calculated from the total N and C excreted and from the known amount of carbohydrate present in the food, assuming, as is usually done, that all of the carbohydrate eaten each day was oxidized.

TABLE I.

ENERGY BALANCE IN PREGNANCY.

(THE FIGURES ARE FOR TWENTY-FOUR HOURS AND REPRESENT THE MEAN OF TWO DAYS FOR EACH WEEK. FOR COMPLETE DATA SEE TABLE III AT END OF PAPER, FIRST PREGNANCY.)

Week.	Weight in kg.	Mean temp. of cage.	Calories of total energy.			Cal. energy per kilogram.	
			In food.	Produced.	Retained.	In food.	Produced.
I	12.29	21.7° C.	662.5	550.6	111.9	53.8	44.6
II	12.24	16.7°	905.6	612.4	293.1	73.4	50.0
III	12.63	18.9°	867.2	572.7	294.5	68.6	45.4
IV	12.70	19.6°	867.2	554.5	312.7	68.2	43.6
V	13.07	23.7°	907.4	543.2	364.2	69.5	41.8
VI	13.33	22.3°	907.4	547.7	359.7	68.1	41.2
VII	13.80	?	907.4	583.1[1]	324.3	66.4	42.3
VIII	14.0	22.8	907.4	628.9	278.5	64.6	44.8

[1] Estimated from VI and VIII weeks.

To speak of the energy production at different stages of the pregnancy first, it may be noted (Table I) that the low result in the first week is due partly to the relatively high temperature as compared with the weeks immediately following, and partly to the low energy content of the food — 53.8 cal. per kilogram. The high result in the second week, on the contrary, is due to opposite conditions in both respects. While it is to be presumed that the total energy production in the fourth week would have been greater than in the third week, had the temperature been the same on the experiment days, it could not have been much greater. The food was the same, and the average temperature, read about once an hour, was only 0.7° lower in the third than in the fourth, yet the daily total for the third week is three per cent more than for the fourth. This confirms the results reported by L. Zuntz[56] and is in accord with Rubner's idea that the total heat production of the embryo as compared with that of the mother does not rise above the zero line until near the middle of the gestation period.[57] Even in the sixth week we cannot be sure that the slight increase over the fifth is not due to the slightly lower temperature. The experiment was interrupted in the seventh week. The figures given for this week are the means of the results for the sixth and eighth weeks. Not until the eighth week, therefore, can we be absolutely certain of an increased energy production. The difference between the sixth and eighth, about 15 per cent, is so great, however, that it is reasonably certain that the seventh week would have shown an increase of about the amount indicated in the table. The absolute increase in oxygen absorption which Magnus-Levy reports for the eighth week over that of the sixth, in his single case, is only 4 per cent. Magnus-Levy does not state whether his subject was on the same diet at the time of the different experiments or whether the experiments were made at the same time of day. Assuming that such was the case, one is inclined to conclude from this single experiment that the increase is much more rapid near the end of gestation in the dog than in the woman. The dog was a well-trained animal and was seen frequently, every one or two hours at most, throughout the respiration period. She lay perfectly still nearly all the time. The difference noted between the sixth and eighth weeks, therefore, is not due to a difference in muscular activity, and since the conditions as regards both the food and temperature were nearly identical, the figures give an accurate picture of the difference in energy production for these periods.

Calculated per kilogram of body weight the metabolism in the eighth week is nearly 9 per cent higher than in the sixth. In Magnus-Levy's case the difference is only 2 per cent.

Other differences noticeable in the last column of Table I are due, as already explained, partly to differences in temperature and partly to differences in the calorific content of the diet.

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[56] ZUNTZ: *Loc. cit.*

[57] See p. 141.

THE METABOLISM JUST BEFORE AND JUST AFTER PARTURITION.

Much more significant than these few figures on the rate of metabolism at different stages of the pregnancy are the results obtained by comparison of the total energy production just before and just after parturition with that of the mother dog alone in sexual rest. These results are shown in Table II. The temperature was the same, within 1° C., on all these days, the diet was exactly the same, and, since the

TABLE II.

ENERGY METABOLISM BEFORE AND AFTER PARTURITION COMPARED WITH SEXUAL REST. RESULTS CALCULATED FOR TWENTY-FOUR HOURS (SEE TABLE III).

FIRST PREGNANCY.

Day from parturition.	Weight in kg.	Temp. of cage.	Calories. Total energy.			Calories. Energy per kg.	
			In food.	Produced.	Retained.	In food.	Produced.
Third before (June 23)	14.5	28.0° C.	907.4	551.3	356.1	62.5	38.0
Parturition (June 26)	....	One puppy born: weight, 280 gm.					
First after (June 27)	14.0 [1]	28.0°	907.4	640.6	266.8	64.8	45.7
SEXUAL REST; AFTER LACTATION.							
Three weeks after parturition (July 15)	13.78	28.1°	907.4	505.3	402.1	66.5	35.9
SECOND PREGNANCY.							
Third before (Dec. 11)	16.86	27.1°	907.4	763.8 [2]	143.6	53.8	45.8
Parturition (Dec. 14)	....	Five puppies born: weight, 1560 gm.					
First after (Dec. 15)	16.14 [1]	27.0°	907.4	1040.4 [2]	−133.0	56.2	65.0

[1] Combined weight of mother and brood.

[2] These figures on recalculation are slightly different from those already published in preliminary form in the Proceedings of the American Physiological Society, This journal, 1909, xxiii, xxxii, and LUSK: Science of nutrition, second edition, 1909, p. 231.

dog was observed to be quiet on all the days which are here compared, we may safely assume that the differences which the table exhibits are really due to the different physiological conditions represented.

From Table III at the end of the paper may be seen how the onset of labor increases the metabolism through the restlessness induced. Hence, in order to determine the effect of the pregnancy alone, it is necessary to select a day before the restlessness is noticeable. The third day previous to parturition being lower than the fourth day, the presumption is that the dog was slightly more quiet on this day, although no difference was observed. The increase in metabolism over that of sexual rest attributable to the pregnancy alone for the single puppy dog is (551.3 – 505.3 =) 46 cal., or 164 cal. per kilogram of puppy dog delivered three days later.

In the second pregnancy the appearance and weight of the dog indicated a larger delivery than from the first pregnancy, and the possibility of comparing the effect of a multiple pregnancy with that of a single was appreciated in time to reproduce exactly the conditions under which the first experiments were carried out. In making up Table II the corresponding days from the two parturitions were selected. As may be seen from Table III, the third day previous shows in this pregnancy a higher energy production than the fourth, as we should expect if the dog were equally quiet on the two days. Since nothing was observed to the contrary, this day may be taken as properly representing the same intensity of metabolism as the third day before the first parturition. The energy production attributable to this pregnancy, therefore, is (763.8 – 505.3 =) 258.5 cal. or 165 cal. per kilogram of puppy dog delivered three days later.[58]

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[58] This figure is based on the assumption that the mother dog's weight and condition as regards fat would have been the same if lactation had been terminated at the same time as after the first pregnancy. It will be noted that the weight on July 15 was almost the same as on June 27 (14.0 — 0.280 (puppy) = 13.72). The mother dog alone on December 15 weighed (16.14 — 1.560 (puppies) =) 14.58 kg. Since the five puppies made a heavy drain on the mother on this first day after parturition, 133 calories having been taken from the mother's own body, it is probable that if the lactation had been terminated at the end of a week and a maintenance diet had been given as before, the weight three weeks (nineteen days, to be accurate) after the second parturition would have been nearly what it was three weeks after the first.

The extra energy production of the pregnancy at or near its culmination therefore is proportional to the weight of the offspring to be delivered (46 cal.: 258.5 cal.: : 280 gm.: 1560 gm. nearly).

It is interesting to observe that the extra metabolism necessary to maintain the embryo (and all accessory structures of the mother's body) at a time when the pregnancy is at its highest phase is very nearly equal to the amount which the new-born of the same weight would theoretically produce (according to Rubner's law of skin area), the first day after delivery, if exposed to ordinary room temperature and if resting. Taking the curve which Rubner gives, on the basis of Hensen's figures, as representing the rate of growth of the guinea pig embryo in a gestation period of almost the same length as that of the dog (sixty-seven instead of sixty-three days),[59] we may calculate that the single puppy which weighed 280 gm. at birth would have weighed 240 gm. three days before birth, and the average weight of the five puppies (320 gm. at birth) would have been 280 gm. three days before. Using Meeh's formula, the metabolism of the single puppy at 240 gm. would be $11.2\sqrt[3]{(240)^2} \times 0.1050 = 45.4$ cal. and of the average puppy of the five at 280 gm. $11.2\sqrt[3]{(280)^2} \times 0.1050 = 50.33$ cal. Thus:

		Cal.
Extra metabolism of a dog pregnant with a puppy of	240 gm. =	46.0
Theoretical metabolism of a puppy of 240 gm. at rest and at room temperature	=	45.4
Extra metabolism of a dog pregnant with five puppies weighing (5 × 280)	1400 gm. =	258.5
Theoretical metabolism of five puppies weighing 280 gm. each at rest and at room temperature	=	251.6

If this law[60] of skin area is applicable to the embryo and the new-born, as Rubner believes it is, we may conclude that the metabolism of the uterus, mammæ, etc., would almost exactly compensate for the difference between the metabolism of the new-born at room temperature and the metabolism of the embryo at the temperature of the mother's body. In other words, the curve of total metabolism of mother and offspring would scarcely suffer any interruption at the birth, if it were not for the muscular activity of mother and offspring.

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[59] Rubner: Archiv für Hygiene, 1908, lxvi, p. 187.

[60] This law has been shown by Hösslin to be applicable to the energy production of locomotion.

That it does, in fact, suffer a very considerable rise may be seen from Table II. The mother and single puppy the first day after parturition show a metabolism of 640.6 cal., an increase of 89.3 cal. in twenty-four hours (or 16 per cent over that of the highest phase of pregnancy, and 135.3 cal. (or 26 per cent) over that of sexual rest. The extra energy produced by the offspring and mother in enabling the former to obtain a foothold under new conditions to which it is subjected after birth is, therefore, three times as great as the extra metabolism necessary to maintain the embryo ***in utero***. In the case of the multiple pregnancy the mother and five puppies produced 276.6 cal. per day (or 36 per cent) more than the third day before parturition, and 535.1 cal. (or over 100 per cent!) more than is produced by the mother dog alone in sexual rest. This shows what an enormous drain the mother's own functions are subjected to in order to provide the energy merely to maintain life in her own tissues and those of her offspring, to say nothing of the material necessary for growth. The extra energy produced by both offspring and mother in enabling the five to obtain a foothold under the new conditions are, however, less than twice as great as the extra metabolism necessary to maintain the five ***in utero***. The extra metabolism of mother and offspring after birth is not, therefore, proportional to the weight of offspring (135.3 : 535.1 = 1 : 4 nearly, while 280 : 1560 = 1 : 5.5).

The economy of the larger family is doubtless due to the fact that the five puppies helped to keep each other warm.

CONCLUSIONS.

1. In a dog pregnant with only one embryo the increase in the total energy production due to pregnancy was not evident until the sixth week of gestation. Between the sixth and eighth weeks an increase of 9 per cent was observed.

2. The total energy production due to pregnancy, measured by the indirect method three days before parturition (in two different pregnancies of the same dog), proved to be proportional to the weight of the offspring at birth.

3. The extra energy production near the end of pregnancy is very nearly equal to the energy requirement of the new-born, when calculated according to Rubner's law of skin area.

4. The extra energy production of mother and offspring the first day after parturition is from two (five puppies) to three (one puppy) times the extra energy production of pregnancy three days before parturition.

TABLE III.

Data for Calculation of the Heat Production.

First Pregnancy.

Week of gestation.	Day of exp.	N in urine.	C in urine N × 0.61.	N in feces.	C in feces N × 6.7.	C of respiration.	Total C excreted.	Total N excreted.	C belonging to			Grams of food-stuffs burned.			Calories of heat produced from			Total heat production.
									Prot. (N×3.28).	C–H (all fed).	Fat (remainder) of C excr.	Prot. (N×6.25).	C–H (44.4% C).	Fat (76% C).	Prot. (× 4.1).	C–H (× 4.1).	Fat (×9.3).	
I	April 30	6.39	3.90	0.54	3.61	47.14	54.65	6.93	22.74	16.87	15.04	43.34	38	19.79	177.6	155.8	184.0	517.4
	May 1	6.91	4.21	0.54	3.61	52.86	60.69	7.45	24.44	16.87	19.38	46.57	38	25.50	190.9	155.8	237.1	583.2
II	7	7.71	4.70	0.60	4.04	56.28	65.02	8.32	27.29	16.84	20.89	52.01	40[5]	27.49	213.2	156.0[6]	255.6	624.8
	8	7.49	4.57	0.60	4.04	55.12	63.73	8.09	26.55	16.84	19.34	50.60	40	25.45	207.4	156.0	236.7	800.1
III	15	7.81	4.76	0.38	2.59	50.32	57.68	8.20	26.90	16.84	13.93	51.27	40	18.34	210.2	156.0	170.5	536.7
	16	7.46	4.55	0.38	2.59	56.01	63.16	7.84	25.74	16.84	20.57	49.05	40	27.07	201.1	156.0	251.7	608.8
IV	21	7.88	4.81	0.29	2.00	51.51	58.32	8.18	26.85	16.84	14.62	51.17	40	19.24	209.7	156.0	178.9	544.6
	22	7.62	4.65	0.29	2.00	53.07	59.72	7.92	25.99	16.84	16.88	49.24	40	22.22	201.8	156.0	206.6	564.4
V	28	8.35	5.09	0.35	2.35	50.35	57.79	8.70	28.54	16.87	12.38	54.39	38	16.29	222.9	155.8	151.4	530.1
	29	8.05	4.91	0.35	2.35	52.54	59.80	8.40	27.58	16.87	15.14	52.55	38	19.92	215.4	155.8	185.2	556.4
VI	June 4	7.92	4.83	0.53	3.61	50.78	59.23	8.46	29.77	16.87	14.58	52.92	38	19.19	216.9	155.8	178.4	551.1
	5	7.92	4.83	0.53	3.61	50.23	58.68	8.46	27.77	16.87	14.03	52.92	38	18.47	216.9	155.8	171.7	544.4
VII	11	7.95	4.84	0.42	2.86	54.12[3]	61.84	8.37	27.47	16.87	17.49[3]	52.36	38	23.01[3]	214.6	155.8	213.9[3]	584.3
	12	7.95	4.85	0.42	2.86	53.70[3]	61.41	8.38	27.48	16.87	17.06[3]	52.37	38	22.76[3]	214.6	155.8	211.6[3]	582.0

VIII	18	7.13	4.35	0.43	2.88	57.46	64.70	7.56	24.80	16.87	23.01	47.27	38	30.28	193.8	155.8	281.6	631.2
	19	7.20	4.39	0.43	2.88	57.16	64.44	7.66	25.12	16.87	22.44	47.87	38	29.53	196.2	155.8	274.6	626.6
IX	22	7.42	4.52	0.54	3.67	51.63	59.83	7.97	26.15	16.87	16.81	49.83	38	22.11	204.3	155.8	205.6	565.7
	23[7]	8.06	4.91	0.54	3.67	50.82	59.41	8.60	28.23	16.87	14.30	53.80	38	18.82	220.5	155.8	175.0	551.3[7]
	24[8]	7.35	4.48	0.54	3.67	55.86	64.02	7.90	25.92	16.87	22.22	49.40	38	29.24	202.5	155.8	271.9	630.2[8]
Labor	25[9]	7.81	4.56	0.54	3.67	74.82	83.05	8.36	27.42	16.87	38.76	52.26	38	50.10	214.2	155.8	465.9	835.9[9]
Partu-rition	26	9.04	5.51[3]	0.50	3.35	[4]	...	9.54	31.31	16.87	...	...	..	...	...	...	...	...
1st day post[1]	27	7.95	4.85	0.50	3.35	57.65	65.85	8.45	27.73	16.87	21.25	52.84	38	27.95	216.4	155.8	268.4	640.6
Sexual rest	July 15	4.57	2.79	0.50	3.35	45.51	51.65	5.07	16.64	16.87	18.13	31.72	38	23.6	130.0	155.8	219.4	505.3
									SECOND PREGNANCY.									
	Dec. 9	5.71	3.48	0.54	3.67	...	...	...	...	...	...	...	...	...	...	...	...	...
IX	10	6.03	3.68	0.54	3.67	65.42	72.77	6.58	21.58	16.87	34.32	41.13	38	45.16	168.6	155.8	419.9	744.3
	11	6.28	3.83	0.54	3.67	67.16	74.67	6.83	22.40	16.87	35.39	42.69	38	46.56	175.0	155.8	433.0	763.8
	14	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Day of partu-rition	15	10.49	6.39	0.50	3.35	92.46	102.20	10.99	36.04	16.87	49.28	68.68	38	64.84	281.6	155.8	603.0	1040.4
	16	7.88	4.81	0.50	3.35	105.10	113.27	8.38	28.51	16.87	67.88	52.43	38	89.32	214.9	155.8	830.6	1201.3

[1] Mother and puppy.
[2] Probably not correct.
[3] Estimated; see text, page 148.
[4] Dog not in resp. cage.
[5] Cane sugar 42.1% C.
[6] (× 3.9 cal. per gram).
[7] Dog perfectly quiet.
[8] Dog restless.
[9] Dog restless 1st stages of labor.

THE INFLUENCE OF INCREASE OF ALVEOLAR TENSION OF OXYGEN ON THE RESPIRATORY RATE AND THE VOLUME OF AIR RESPIRED WHILE BREATHING A CONFINED VOLUME OF AIR.

BY THEODORE HOUGH.

[*From the Physiological Laboratory of the University of Virginia.*]

THE respective rôles of oxygen and of carbon dioxid as regulators of the breathing movements have been the subject of repeated investigation since the publication of Rosenthal's work in 1862. The conclusion of this investigator[1] that the stimulus to the automatic action of the respiratory centre is the oxygen deficiency of the blood flowing through the centre has failed of confirmation by subsequent work. On the contrary, results such as those of Zuntz[2] have shown that whereas the percentage of oxygen in the inspired air may be diminished by one half the normal without greatly influencing the volume of air breathed, the presence of 1 per cent of carbon dioxid increases the minute volume by more than 20 per cent. Finally, the investigations of Haldane[3] and his co-workers have brought to light the relation between the breathing movements and the alveolar tension of carbon dioxid, and have led to the theory, so ably supported on experimental grounds by Haldane, that carbon dioxid is the sole stimulus of the automatic action of the respiratory centre. According to this theory, deficiency of oxygen influences the centre only through the consequent production of the intermediate acid products of incomplete oxidation. The presence of these in the tissues or blood lowers the combining power of lymph or blood for the carbon dioxid produced by the cells and hence increases the tension of this gas in the respiratory centre.

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[1] ROSENTHAL: in HERMANN'S Handbuch der Physiologie, 1882, iv, pp. 261 foll.

[2] ZUNTZ: Archiv für Physiologie, 1897, p. 379.

[3] HALDANE and PRIESTLEY: Journal of physiology, 1905, xxxii, p. 225.

During the past year I have been engaged in experiments of various kinds concerned with the questions thus presented, and I propose to report in the present paper a series which shows the effect of increased oxygen tensions on the rate of the respiratory movements and minute volumes of air breathed in the dyspnœa produced by breathing a confined atmosphere.

EXPERIMENTAL PROCEDURE.

The essential features of the experimental procedure are shown in Fig. 1. The subject of the experiment breathes a closed volume (approximately 30 litres) of air through a mouthpiece, *A*, provided with valves *H* and *C* (see also Fig. 2), for separating the inspired and expired air. The glass tubing and thick-walled rubber tubing connections are 15 mm. (⅝ inch) inside diameter. The expired air passes from the expiratory valve into the upper portion of a 20-litre glass bottle, *D*, while the tube to the inspiratory valve passes off from the bottom of this bottle. On the way from the bottle to the mouthpiece is a side tube, *E*, to the air chamber, *F*, of a modified Gad's aeroplethysmograph.[4] This air chamber, which thus forms part of the closed volume of air breathed, is suspended from the centre beam of a truss (to secure rigidity of the lever) which moves on the knife edge, *K*. The same centre beam carries the adjustable counterpoise, *L*, and ends in the writing point, *M*, which records on the kymograph drum. Since the chamber of the aeroplethysmograph is the only movable part of the closed air space, it is evident

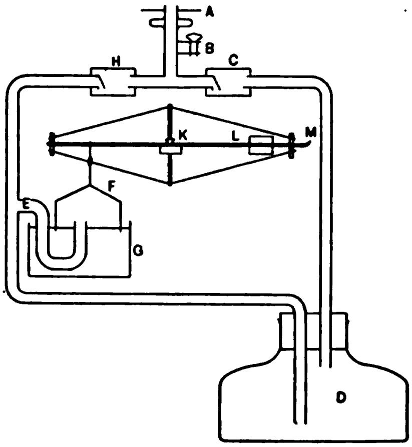

FIGURE 1. — Diagram of the essential parts of the apparatus. *A*, mouthpiece with lip and teeth flanges; *B*, side tube for taking specimen of alveolar air; *C*, expiratory valve; *D*, 20-litre bottle; *E*, side tube to air chamber, *F*, of the aeroplethysmograph; *G*, the water seal; *H*, inspiratory valve; *K*, knife edge; *L*, counterpoise; *M*, writing point.

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[4] GAD: Archiv für Physiologie, 1879, p. 181. See also NAGEL: Handbuch der Physiologie des Menschen, i, p. 17.

that each inspiration will be recorded as an upward and each expiration as a downward stroke of the writing point. The instrument is easily calibrated by closing the mouthpiece, *A*, and running into *D* measured volumes of water from a pressure bottle.

The valves. — The separation of the expired from the inspired air is secured by the use of the valve shown in Fig. 2. The side arms of the T-shaped mouthpiece are fastened by rubber stoppers into the valve chambers *B* and *D*. Each valve chamber is provided with a circular membrane valve made of fairly thick sheet rubber, pinned above into the projecting rim of the rubber stopper, which thus serves as the valve seat. Such membrane valves are perfectly efficient except in cases of slight pressure differences. To care for these the Müller's water valves, *A* and *E*, are placed beyond the membrane valves, the inlet tube of each valve dipping down not more than 1 mm. below the surface of the water. In addition to caring for slight differences of pressure, these water valves are useful to indicate the least incompetency of the membrane valves, as the water in them should never rise more than 2 or 3 mm. when the membrane valve is working properly. The side tube *H*, closed by a rubber stopper, is to receive the water condensed from the expired air, and also to facilitate cleaning the valve. The water valve is provided with the tubes *F* and *G* by which the water may be renewed without disturbing the stopper and also for the accurate adjustment of the level of the water. The side arm may also be used in the inspiratory membrane valve, *B*, and the arrangement for renewing and adjusting the level of water may be used in the inspiratory water valve, *A*; but they are not so necessary here, since water does not condense in these valves.

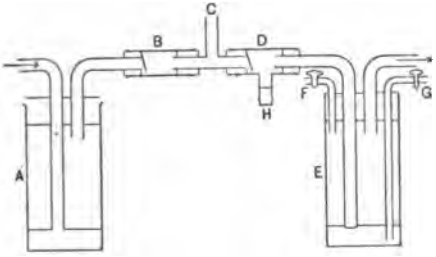

FIGURE 2. — The valves for separating inspired and expired air. *A*, inspiratory water valve; *B*, inspiratory membrane valve; *C*, tube from mouthpiece; *D*, expiratory membrane valve; *E*, expiratory water valve; *F* and *G*, tubes for renewing and adjusting level of water in water valves; *H*, side arm on membrane valve to collect condensed moisture.

Details of construction of the aeroplethysmograph. — The air chamber is made of the thinnest tin plate and is 30 cm. in diameter and 22 cm. high to the base of the conical top (Fig. 1). It is attached to the centre beam of the truss by a light adjustable clamp in which it swings freely. The centre beam of the truss is made of 5 mm. ($\frac{3}{16}$ inch) steel rod, the upright beam is of 8 mm. ($\frac{5}{16}$ inch) brass tubing, and the other four beams are of 3 mm. ($\frac{1}{8}$ inch) brass rod. The length of the centre beam is 70 cm., and the length of the upright beam 30 cm. The air chamber is closed below by a water seal, and the whole — truss and chamber — can be lifted off the knife edges when not in use.

Errors of friction, inertia, and buoyancy in the aeroplethysmograph. — As to the possible sources of error in the recording instrument, the friction at the knife edges is negligible; that of the chamber as it rises and sinks in the water, while greater, is still slight. It may be considerably reduced by painting the immersed parts with a very thin coat of paraffin.

The inertia of the system for respiratory rates not exceeding twenty-five deep respirations per minute is also negligible. This has been tested by suddenly stopping off the tube leading to the air chamber while the lever is moving with the maximum speed attained in our experiments. In all cases the lever stopped almost instantly, the error introduced into the measurement not exceeding 10–20 c.c. Such speeds of the writing lever occur only in very rapid respirations of over 1500 c.c. in volume. Hence the greatest possible inertia error can hardly exceed 1 per cent. With respirations of ordinary rate and depth (600 to 1000 c.c.) the error is far less. Moreover, even in very deep and rapid breathing, there is usually a marked diminution of speed at the end of both inspiration and expiration, and this alone virtually does away with the inertia error, so far as measurements of each respiratory volume are concerned.

Finally, there is the theoretical error from the change in the buoyancy of the water as the air chamber rises and sinks. That this is very slight, indeed entirely negligible, is shown by the results of calibration of the instrument, the rise (27 mm.) of the writing point caused by a litre change of volume varying by less than a quarter of a millimetre for the highest and lowest positions of the air chamber.

Sources of error in the method as a whole. — Three sources of error are inherent in the method. The first is due to the rise of the temperature of the confined air. The magnitude of this error will obviously vary with the external atmospheric conditions, *i. e.*, with the rate of heat loss from the contained air. As a matter of fact I have never seen a change of more than 2° C. in the temperature of the air in the large bottle (*D*, Fig. 1), and this maximum change is always reached in three or four minutes. This would lead to a depression of the writing point of about 6 mm. (= 220 c.c.). The ordinary volume of air respired in three minutes is about 27,000 c.c. Hence for this period of expansion there could not be introduced more than the maximum error of +1.1 per cent for the volume measurements. Ordinarily the rise of temperature is much less than a degree, and it may be safely asserted that the error from this source seldom exceeds a fraction of 1 per cent and that it is always confined to the first few minutes of the experiment. The amount of this error is, moreover, observed to be independent of the presence of ordinary air or of air containing excess of oxygen in the system.

The second source of error is due to the elasticity of the air used as a medium of transmission to the recording instrument. The error cannot be entirely eliminated; it can only be kept down to the minimal amount by the use of sufficiently large tubes. In our experiments the conducting tubing was 15 mm. (⅝ inch), inside diameter, a size which offers very little resistance. Moreover, the slowing at the end of each inspiration and expiration already referred to in discussing the inertia error of the recording apparatus would act here also to eliminate the error from the elasticity of the air, so far as volume measurements of each inspiration are concerned, and it is only with these that we are concerned in the present paper.

A third source of error renders the tracing somewhat inaccurate as a record of the exact volume change of the thorax, although it does not affect the determinations of minute volumes or rates. It arises from the fact that, the respiratory quotient being usually less than unity, the carbon dioxid returned to the system does not replace, volume for volume, the oxgyen removed. The expiratory tracing would thus not return to the starting-point when the thorax had exactly returned to the position it had at the beginning of inspiration. In other words, there would be a rise of the line representing the initial expiratory size of the thorax. It is clear, however, that this influences only the accuracy of the expiratory tracing, while our measurements of volume are made on the inspiratory tracing.

I am inclined to think that the most accurate records of respiratory volume would be obtained by a combination of the aeroplethysmograph above described with the method of the pneumatic cabinet.[5] The tube passing to the air chamber of the recording apparatus could be chosen large enough to offer no resistance to the air, and the error due to changes of temperature within the cabinet readily obviated by having the interior of the chamber connected with the exterior through a very fine capillary tube. I am planning such an instrument for other work; but for many experiments, such as those described in this paper, the greater trouble involved in the use of such a method is not repaid by the very slight increase in accuracy attained.

The mouthpiece. — A very important part of all such apparatus is a mouthpiece which can be kept fitted air-tight in the mouth. That used in these experiments consists of a hard rubber tube with a flange which can be pressed back against the lips. It gave little trouble, but at times leakage of air could not be prevented, especially in those untrained to the instrument. Since these experiments were completed, the mouthpiece has been made practically perfect. The tube of the mouthpiece is drawn through a small hole in the centre of a piece of flexible sheet rubber, and the latter is tied firmly around the tube about 5 mm. from the flange. The tube is placed in the mouth so that the sheet rubber is between the lips and teeth. With this mouthpiece leakage is virtually impossible.

RESULTS.

The experimental results given in this paper are all from the same subject of experiment. I am at present engaged, with the assistance of two of my students, in a study of the variation in respiratory response to dyspnœic conditions identical with those of the present series. The results of these investigations will be published shortly, but their publication may be anticipated by saying that while there are very interesting variations in the individual response to dyspnœic conditions, for each individual the curves of *minute volumes* of air breathed under the experimental conditions above described are remarkably uniform, and this is true despite considerable variations in rate. So constant is this curve that any constant change in it produced by changing a given condition of the experiment is significant. I could wish that the time required for such work did not at present prohibit repeating the experiments with other subjects; but the fact that they cannot be so repeated is my justification for publishing the perfectly clean-cut results which I have obtained on myself.

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[5] SCHENCK: TIGERSTEDT's Handbuch der physiologischen Methodik, ii, 2, p. 35; also NAGEL: Handbuch der Physiologie, I.

The tracing reproduced in Fig. 3 is typical of the experiments where the subject (T. H.) breathes a closed volume (about 30 litres) of atmospheric air or of air with an increased percentage of oxygen. It shows an increase, at first gradual and then more rapid, in the depth of the breathing movements without any increase in rate until about the end of the eighth minute, when the rate suddenly changes. Let me repeat that this type of tracing is by no means characteristic of all subjects of experiment, although it has been observed in others. Some men give a gradual increase in rate from the first, and when this is the case the depth may gradually increase, or remain constant, or even decrease. As stated above, these variations will form the subject of a later paper.

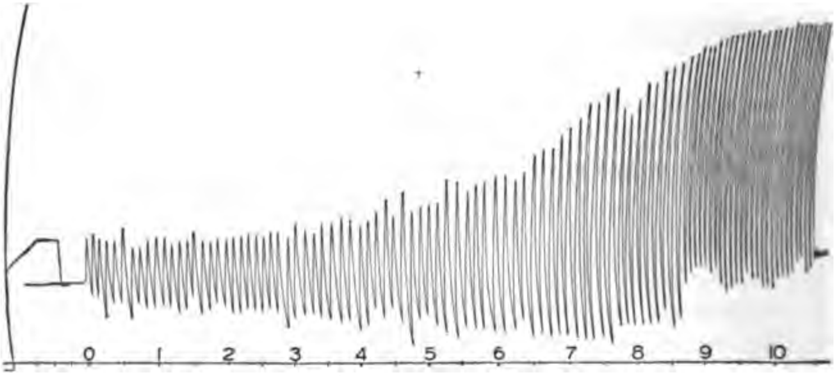

FIGURE 3. — One third the original size. Typical aeroplethysmographic tracing from the subject of these experiments. Time record in half minutes.

The record was always started on the beginning of the minute, the point of the time pen having been adjusted to coincide with the line traced by the aeroplethysmograph with the drum at rest. The latter line was always obtained at the outset for the entire length of the lever stroke, and so furnished the dividing lines between minutes on the tracing. The tracing having been thus divided into its separate minutes, the length of each inspiratory stroke was measured with the calibration scale. When the minute line divided a whole respiration between two minutes, the fraction belonging to each minute was estimated (usually with the aid of a hand lens) and the total inspiratory volume accordingly divided between the two minutes. By this means also the rate per minute can be given in tenths instead of entire respiratory movements. Attention to these simple precautions of measurement greatly contributes to the regularity of the curves obtained.

The influence of increased percentage of oxygen in the air breathed upon the minute volume and the rate in successive minutes are shown in Tables I and II. Six experiments with air and six with excess of oxygen (60 to 80 per cent) are given. These were chosen at random for counting out from a larger number of experiments. The oxygen was obtained from an "Oxodium" generator, being formed by the action of water on sodium peroxid.[6]

These results show that when the respired air contains a large excess (60 to 80 per cent) of oxygen, both the minute volumes of air respired and the rate show a distinct reduction. Moreover, on comparing the percentage reductions of minute volume and of rate (Table III), we find that the reduction in volume, is relatively greater than that of rate, thus showing that not only the rate but also the depth of each respiration is reduced.

In addition to this main result two other facts may be recorded. It will be remembered that in the subject of these experiments there is a remarkably sudden increase of rate after eight or more minutes of exposure to the dyspnœic conditions of these experiments. With excess of oxygen, this change occurred from one to three minutes later than with air. I have also collected and analyzed samples of the alveolar air, by the Haldane method, at the time of this change of rate, and find that the percentage of carbon dioxid at the change of rate is distinctly higher in the experiments with oxygen than in those with air.

DISCUSSION OF RESULTS.

The results of these experiments show an unmistakable influence of increased alveolar oxygen tension on the work of the respiratory centre, not only under the more pronounced dyspnœic conditions of the eighth and following minutes of our experiments, but, what to the writer is even more striking, during the very first minute. It is not my purpose

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[6] Fully described by BENEDICT: This journal, 1909, xxiv, p. 361.

TABLE I.

MINUTE VOLUMES OF INSPIRED AIR IN LITRES.

Date.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AIR.														
April 18, I . .	8.11	8.73	8.86	10.48	12.14	15.12	20.53	26.48	34.37	44.58	48.71	....	....	....
March 26, II . .	8.25	8.66	9.91	10.79	12.78	16.44	19.32	23.45	30.60	36.00	44.41	....	....	....
April 1, II . .	8.62	8.92	10.00	11.33	13.99	15.46	21.89	26.55	35.29	44.82	....	....	....	....
April 12, I . .	9.40	9.46	10.13	10.34	12.78	14.80	18.91	23.61	29.12	36.59	41.75	48.50	....	....
April 20, I . .	8.18	9.10	10.88	11.58	13.29	15.51	22.74	25.66	30.87	41.70	47.39	61.49	....	....
April 19, I . .	10.08	11.69	11.04	13.30	15.03	16.76	21.34	26.36	32.12	41.73	50.34	....	....	....
Average . . .	8.74	9.43	10.14	11.30	13.37	15.68	20.79	25.35	32.06	40.90	46.52	55.05	....	....
OXYGEN EXCESS.														
April 20, II . .	6.51	7.48	7.17	9.04	8.93	11.44	13.87	16.15	19.44	25.89	35.92	31.18	36.89	42.27
April 12, II . .	7.70	7.93	8.59	9.08	9.51	11.40	12.40	14.06	17.33	22.56	27.62	36.06	37.28	40.79
April 18, III .	6.97	7.90	9.73	8.79	12.53	13.60	14.72	18.34	21.52	22.75	32.42	35.56	39.79	43.27
April 10, II . .	7.93	8.18	9.28	9.26	10.56	13.00	13.79	16.68	20.16	23.87	28.55	34.83	39.44	38.07
April 17, I . .	8.13	8.74	8.62	9.00	11.77	12.72	14.82	20.74	23.65	31.26	31.61	36.16	37.49	44.31
April 10, I . .	9.31	9.46	10.28	10.79	12.02	12.95	14.44	16.88	23.57	28.17	29.18	35.41	48.00	....
Average . . .	7.75	8.21	8.95	9.35	10.89	12.52	14.01	17.14	20.95	25.75	30.72	34.87	39.82	41.34

RESPIRATORY RATES PER MINUTE.

Date.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
AIR.														
April 18, I . .	10.0	9.0	8.2	8.7	8.2	7.8	7.8	7.3	9.2	12.6	12.4	...	...	...
March 26, II . .	10.0	10.0	11.0	11.0	11.0	13.0	12.0	12.0	11.0	11.0	15.0	...	...	...
April 1, II . .	13.0	12.0	13.0	13.0	14.0	13.0	12.0	10.0	11.0	17.0	...	...	...	...
April 12, I . .	10.0	10.5	10.5	10.0	10.8	11.2	11.0	10.7	9.0	11.3	15.0	16.0	...	...
April 20, I . .	9.5	10.1	10.6	9.8	8.8	8.3	10.0	9.2	9.1	11.5	15.2	18.5	...	...
April 19, I . .	11.3	11.5	9.2	8.3	7.5	8.3	8.2	8.2	10.4	12.7	13.9	...	...	...
Average . . .	10.6	10.5	10.4	10.1	10.05	10.1	10.2	9.6	9.9	12.7	14.3	17.3	...	...
OXYGEN EXCESS.														
April 20, II . .	9.5	10.2	8.4	8.9	6.6	5.4	5.0	5.0	5.3	6.8	11.8	10.6	11.9	13.0
April 12, II . .	8.8	9.4	9.3	9.0	8.3	8.7	8.1	7.4	7.5	7.4	7.5	11.6	12.0	12.7
April 18, III .	9.3	8.7	8.9	7.1	7.0	6.3	6.3	6.3	7.1	6.6	9.2	11.7	14.1	14.2
April 10, II . .	10.0	10.1	10.0	9.0	9.0	9.6	8.0	7.8	8.0	8.4	8.2	10.6	15.0	14.0
April 17, I . .	10.7	10.1	9.2	8.6	8.2	7.8	6.6	7.8	7.5	9.3	11.0	11.9	11.8	13.0
April 10, I . .	11.0	11.0	11.0	11.0	11.0	10.0	9.0	9.0	9.0	9.0	8.0	12.0	17.0	15.0
Average . .	9.88	9.92	9.47	8.93	8.35	7.97	7.17	7.22	7.4	7.92	9.28	11.4	13.63	13.65

to discuss at any length the theoretical bearing of this fact. Previous to my personal investigation of the subject, I had supposed that, since the usual oxygen supply of the blood is much more than sufficient to meet the respiratory demands of the tissues, excess of oxygen over the normal would not influence the tissues, so long as this excess does not reach the proportions which Paul Bert showed to have toxic effects. It is clear enough that breathing an atmosphere containing 60 to 80 per cent of oxygen can only slightly increase the total quantity of oxygen in the blood, since it can only treble or quadruple the small fraction dissolved in the plasma. In other words, the oxygen content of 100 c.c. of arterial blood may have been raised in our experiments from 19.393 c.c. of oxygen to 20.179 c.c. or 20.582 c.c. Yet our figures show that this small increase reduces the minute volume of air breathed in the first minute from 8.74 to 7.75 litres. We can hardly doubt that this effect is due to the increased oxygen tension of the plasma.

TABLE III.

Percentage Reduction of Rate and Minute Volumes in the Oxygen Experiments compared with Air Experiments.

Minute.	Rate.	Minute volumes.	Minute.	Rate.	Minute volumes.
1	6.9	12.5	7	29.7	32.6
2	5.5	12.9	8	23.7	32.3
3	8.9	11.7	9	25.2	34.0
4	11.9	17.2	10	37.0	37.0
5	17.0	19.0	11	35.0	34.0
6	29.0	20.0	..	....	....

As stated in the introduction to this paper, every increase of our knowledge of the relation of the gases of the blood to the automatic action of the respiratory centre increases the importance of the carbon dioxid as the exciting cause of its activity. Of the correctness of this view personally I have no doubt, not only from my reading of the literature but from my own (unpublished) experiments. The only question is whether carbon dioxid is, as Haldane believes, the sole exciting cause. It is easy on Haldane's theory to explain the results given above.

We can imagine that the arterial blood, arriving at the centre with three or four times its normal tension of oxygen in the plasma, would at once, on reaching the capillaries, begin to discharge its oxygen very rapidly into the tissues, without waiting for the longer process involved in the dissociation of its oxyhæmoglobin; and that this dissociation of the oxyhæmoglobin becomes more effective and important as the blood passes along the capillaries, where its tension of carbon dioxid and perhaps its temperature [7] is increased. It is thus possible that the increase of oxygen tension in the blood may result in a considerable increase of oxygen tension in the lymph, a far more effective supply of oxygen to the tissues, and hence to the production of considerably less of intermediate acids. Under these circumstances the carbon dioxid produced by the cells would be more rapidly combined with the bases of the lymph and so the tension of this gas in the tissues materially lessened. I know of no facts which are inconsistent with this explanation, and it seems to me to be at present the best working hypothesis; but, like all working hypotheses, it requires confirmation by further tests.

The chief interest of these results to me lies in the indication they afford of the influence of breathing air rich in oxygen on the physiological condition of the tissues, for it seems fair to regard the behavior of the respiratory centre as indicative of the conditions which must obtain under the conditions of our experiments in every capillary region of the body. In other words, the significance of the increased oxygen content of the blood when one is breathing pure oxygen lies, not in the gross increase, but in the increased oxygen tension which it carries with it. What we may term the attack of oxygen on the tissues is thereby rendered much more immediate and effective.

There is also in our results the obvious suggestion that the amount of oxidation in the body is not determined, even under ordinary conditions, by what is usually termed the "need" of the tissues for oxygen, but that the pressure of oxygen in the tissues is also an important factor in the result. It may well be that the influence of oxygen we have observed in our experiments is the same action which, when taking place under three or more atmospheres' pressure of the gas, produces toxic effects. I know of no experiments sufficiently controlled to give definite information as to the influence of increasing the oxygen tension of the blood on the oxidations of the body; and in the absence of these it would be unprofitable to do more at this time than to question the soundness of the usual view that the consumption of oxygen by the cell is determined entirely by the cell and is independent of the quantity of oxygen provided by the blood.

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[7] Barcroft and King: Journal of physiology, 1909, xxxix, p. 374.

SUMMARY.

1. A method is described of obtaining very accurate records of the rate and depth of the breathing movements.
2. When one breathes a confined atmosphere of about 30 litres in volume and containing an initial percentage of from 60 to 80 per cent of oxygen, the respiratory rate and minute volumes of air breathed are distinctly lower than when the initial atmosphere is ordinary air; and this effect is shown, not only in the later periods of distinctly labored breathing, but from the very first.
3. Increase of oxygen in the air breathed distinctly modifies the respiratory condition of the tissues, if we can take the behavior of the respiratory centre as an indication of this condition.

THE RELATION OF PTYALIN CONCENTRATION TO THE DIET AND TO THE RATE OF SECRETION OF THE SALIVA.

By A. J. CARLSON and A. L. CRITTENDEN.

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I. THE RELATION OF PTYALIN CONCENTRATION TO THE DIET.

A. **In Man.**

THE ptyalin concentration in the various salivas was tested by the starch method. There was mixed with 1/10 or 2/10 c.c. of saliva 25 c.c. of 1 per cent boiled starch, and the rate of clearing of the starch solution, the rate of disappearance of the blue starch color on addition of iodine, and the rate of disappearance of the erythrodextrin determined. In this way we have three checks on the relative rate of digestion of the different saliva samples, and when all three correspond we are pretty certain to have an actual difference in ptyalin concentration. The advantages of this method are its simplicity and its relatively quick application.

At the New York meeting of the American Physiological Society in December, 1906, Nielson[1] reported that forced diet of carbohydrates or sugar in man, even for a period of three or four days, greatly increases the ptyalin concentration in the mixed saliva. The same author had previously reported similar observations on the dog.[2] Nielson's results on the dog have not only not been confirmed by other observers (Mendel and Underhill,[3] Garrey,[4] Carlson and Ryan[5]), but it has been shown that the saliva of dog and cat contains no ptyalin, but only traces of the blood and lymph diastases that apparently get into the saliva along with other constituents of the body fluids.

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[1] NIELSON and LEWIS: Journal of biological chemistry, 1908, iv, p. 501; NIELSON and SCHEELE, *Ibid.*, v, p. 331.

[2] NIELSON and TERRY: This journal, 1906, xv, p. 406.

[3] MENDEL and UNDERHILL: Journal of biological chemistry, 1907, iii, p. 135.

[4] GARREY: Proceedings of the American Society of Biological Chemists, 1907, i, p. 49.

[5] CARLSON and RYAN: This journal, 1908, xxii, p. 1; CARLSON and LUCKHARDT: This journal, 1908, xxiii, p. 148.

1. In the fall of 1908 the diastatic power of the parotid saliva of three individuals (A. J. C., C. B., A. L. C.), designated for convenience A, B, and C, was compared daily for a period of ten days. The saliva of A was uniformly slightly stronger than that of C and considerably stronger than that of B. At the end of the ten-day period B and C were put on an exclusively vegetarian diet for ten days, that is, meat was excluded and carbohydrates greatly increased, while A continued on the ordinary mixed diet. The diastatic power of the parotid saliva was tested daily in the three individuals, and *at no time was there an increase in ptyalin in the saliva of B and C as checked against A.* The salivas in these experiments were collected by the insertion of a glass cannula into the opening of Stenson's duct and a weak acetic acid in the mouth was used as a stimulus. The acid used throughout the experiment was of the same strength, and the time of collection of saliva in the three individuals was the same with reference to time of day and length of time after meals.

2. It occurred to us that if no difference in ptyalin concentration can be produced by diet alterations over a period of ten days, possibly prolonged vegetarianism might reveal such differences. Consequently the diastatic power of the parotid saliva of a man who for four years had been a consistent vegetarian was checked for seven days against that of A and C. It showed uniformly less concentration than that of A and practically the same as that of C. The subject was middle-aged and in good physical condition.

3. The parotid and mixed saliva of a boy of fourteen who might be termed a "congenital vegetarian," having practically never eaten meat, was checked against the corresponding salivas of A and C. The parents of this boy report that from early childhood the boy had refused meat or meat products in food, and that his food consists mainly of vegetables, cereals, bread and milk. These tests were continued daily for six days. The diastatic power of the boy's saliva was uniformly less than A and about the same as C. The boy was in every respect normal and in good physical condition.

Thus, contrary to Nielson's results, there is no evidence that the ptyalin concentration in the parotid or the mixed saliva is appreciably increased in man, even after years in which meats are excluded and carbohydrates greatly increased.

B. **The relation of ptyalin concentration to diet in other animals.** — It is conceivable that while shorter periods of meat exclusion and carbohydrate increase in the diet of man may not effect an increase in the ptyalin, generations of vegetarianism might be effective. This could be tested on the saliva of orthodox Hindoos who abstain from meat, and possibly on Chinese coolies who in their native country subsist mainly on rice; but we were not able to secure any of this material. It is obvious, however, that the experiment has been carried out in nature on a large scale in the case of the lower mammals.

1. *Carnivora.* — It has previously been shown that dog and cat saliva contains no ptyalin, and that the slight diastatic power of the saliva of these animals is due to the admixture of blood and lymph diastases. To this list of carnivora can now be added the fox. We collected pure parotid saliva in five foxes. This can easily be done in the fox by insertion of a cannula in the opening of Stenson's duct, or by holding a suitable spoon below the opening of the duct and stimulating the nerve endings in the mouth with weak acetic acid or sand. The results of the test on the fox are the same as in the case of the dog and the cat. The fox parotid exhibits a weak diastatic action, but very much weaker than the blood or the lymph of the same animal.

2. *Herbivora.* — If there is any adaptation of ptyalin to the nature of the food, this ought to be revealed in the monkeys, whose food for ages probably has contained little if any meat. We tested the diastatic power of the parotid and the mixed saliva in seven monkeys of different species and of different ages. All were in good condition. Our results showed conclusively that the ptyalin concentration of the saliva of monkeys, so far from being greater, is the same or even less than in man.

It is well known that the parotid saliva of the rabbit is relatively rich in ptyalin, and our comparative measurements show that the concentration is the same or slightly greater than in the human parotid saliva.

In 1908 one of the authors, working together with Dr. Woelfel, had occasion to test the diastatic power of the pure parotid saliva in a goat; he was surprised to find that it exhibited practically no solvent action on boiled starch. That particular goat, however, was in poor condition at the time the saliva was collected. We have now tested the saliva of five other goats of different ages and all in good physical condition, and obtained similar negative results. In one of the goats a temporary fistula had been established in one of Stenson's ducts and the saliva collected while the animal was normally eating; in the other four animals the mixed saliva from the mouth was used for the tests. When 1 c.c. of the goat saliva, parotid or mixed, is added to 25 c.c. of starch (1 per cent) and the mixture placed in a thermostat at 38°, there is no appreciable clearing of the starch solution in twenty-four hours.

In connection with other work one of the authors together with Drs. Becht and Greer had occasion to collect pure parotid saliva from two horses, secreted after pilocarpin injection. The diastatic power of these salivas was tested, incidentally, with negative results. These horses were, however, old and not in good physical condition, and it occurred to us that the absence of ptyalin might be due to the poor condition of the animal, to age, or to the secretion of the ferment in an inactive form, the activation taking place in the mouth. For that reason we collected the mixed saliva from the mouth of twelve horses, all of which were in the best physical condition, but the tests of the saliva from this group of horses also were negative. One cubic centimetre of the saliva added to 25 c.c. of boiled starch (1 per cent) and kept at 38° C. exerted practically no solvent action in twenty-four hours.

It would therefore seem that ptyalin is absent in the saliva of many and probably all of the carnivora. And in the herbivora it may either be absent or present. The absence of the ptyalin in the carnivora and its presence in the rodents and primates may suggest adaptation to the diet, but the absence of ptyalin in some of the herbivora nullifies such a conclusion. Moreover, the ptyalin concentration of the saliva in monkeys is not greater than that in man, while the diet of monkeys is certainly more strictly vegetarian, on the whole than that of man.

The significance of the presence or absence of ptyalin in the saliva in the different groups of mammals presents some interesting biological problems. The fact that ptyalin acts very slowly on raw starch would seem to make the ferment of little digestive value in all mammalian groups with the exception of man, yet it is present in the monkeys and the rodents. It is conceivable that in the ruminants the ptyalin may be secreted in an inactive form and activated in the stomach, but we have not had the chance to test this possibility. In horses and goats there is certainly no activation in the mouth. And in non-ruminating herbivora activation of ptyalin in the stomach would have no physiological significance because of the rapid destruction of the ptyalin by the hydrochloric acid. We do not wish to be understood as holding that the ptyalin-producing processes are accidental or have been evolved without any relation to the nature of the food; we must have data from all the mammalian groups before we can determine whether the absence of ptyalin signifies atrophy or incipient evolution.

II. THE RELATION OF THE PTYALIN CONCENTRATION TO THE RATE OF THE SECRETION OF SALIVA.

The saliva is the only digestive juice whose secretion can be studied under absolutely physiological conditions. Little or no work has been done in man on the ptyalin concentration in the saliva secreted under different conditions of appetite, emotional states, or in response to different stimuli in the mouth, because of the difficulty of securing the saliva free from admixture with the substances used for stimulation, and because the ordinary laboratory animal, the dog, as we have seen, has no ptyalin in the saliva. The rabbit's parotid secretes ptyalin, but the rabbit is a small animal, the salivary glands are correspondingly small, and it is difficult to get enough parotid saliva for physiological tests without fatiguing the gland.

It is relatively easy to collect pure parotid saliva in man by the insertion of a cannula into Stenson's duct. But this cannula must be held in place by an attendant, the subject's head must be held relatively rigid, and only very limited masticatory movements can be executed without displacing the cannula. Moreover, in the case of problems involving accurate determination of secretory rate, this method is rather unsatisfactory because a cannula of the size that will easily slip into the duct will usually permit some saliva to escape between the edges of the duct and the cannula itself. It is perfectly obvious that this method does not permit the collection of a pure parotid saliva during the mastication of an ordinary meal. We attempted to devise a cannula that would be suitable for the human parotid. An illustration of this cannula as constructed by us is given in Fig. 1. The principle is simply this: to surround the opening of Stenson's duct with a metal cup which communicates with the exterior by means of a metal tube; surrounding this inner cup and fixed to it is an outer larger one communicating with the exterior by means of another metal tube. The cannula is held in place by a vacuum produced in the outer cup by means of suction, the cannula being pressed flat against the sides of the cheek. The side of the cannula facing the mouth is rounded off and the thickness of it so graded as to interfere as little as possible with the movement of the jaws in mastication. Such a cannula can be applied over the duct and held in place by suction as indicated, and if the gland is secreting the saliva will flow out through the metal tube connected with the inner cup. But in our experience this apparatus did not prove very satisfactory because masticatory movements will sooner or later dislodge it, and in no case did we succeed in keeping the cannula fixed in place throughout the whole period of mastication of an ordinary meal. In most of our work, therefore, we had to fall back on the old method of collecting the parotid saliva by inserting a cannula directly into the duct opening. In monkeys the permanent salivary fistula, as used by Pawlow in the dog, is applicable.

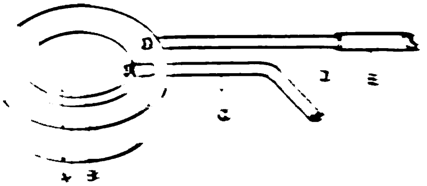

FIGURE 1. — Cannula for collecting the parotid saliva, right side, in man. 1, cup receiving the opening of Stenson's duct. 3, outer cup for fixation of the cannula by means of suction. 2, outlet tube for the saliva. 4, 5, metal tube with rubber tubing for the production of a vacuum in cup 3.

2. In man weak acetic acid in the mouth appears uniformly a more efficient stimulus to secretion by the parotid than mechanical stimuli, such as dry sand, crackers, flour, or cotton.* This has been tested in many individuals and in some a great number of times with the same results. Within limits the stronger the acid the greater the rate of secretion. This difference between efficiency of mechanical and acid stimuli is probably only one of intensity of stimulus. It is obvious that a chemical like acetic acid will get in contact with a greater number of

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* This appears to be contrary to PAWLOW's well-known results on dogs, showing apparent adaptation of the saliva to the nature of the stimuli in the mouth. But Pawlow's results have not been confirmed. *Cf.* POPIELSKI, Archiv fur die gesammte Physiologie, 1909, cxxvii, p. 433.

nerve endings than a substance like dry sand or flour when applied to the same area.

We have made a number of determinations of the relation of concentration of the human parotid saliva to the rate of the secretion of the saliva, and find that it is the same as in the lower mammals, namely, within limits the concentration of the saliva increases with the rate of the secretion.

2. It has been shown by Carlson and Ryan[7] that the ptyalin in the parotid saliva of the rabbit varies directly with that of the organic solids, at least in the case of gland anæmia and on stimulation of the cervical sympathetic. The behavior of the ptyalin concentration in the relation to the secretory rate was not determined, but in view of these findings we would expect the ptyalin concentration to increase with the secretory rate in the same way as the organic solids increase with the secretory rate in the rested gland. We endeavored to answer the question by direct experiments on man, rabbits, and goats (before we found out that the goat saliva contained no ptyalin).

In man, in the case of individuals who respond readily with varying secretion rates to stimuli of varying strengths, *the saliva that is secreted the fastest exhibits the greatest digestive power*. We used different strengths of acids alternately, or sand and acids alternately. The slowly secreted saliva obtained on placing sand in the mouth contains less ptyalin than that secured on stimulation by weak acetic acid. The acetic acid stimulus may be graded in strengths 1, 2, 3, and applied to the mouth consecutively, beginning either with the weakest or the strongest, and the parotid saliva obtained will exhibit corresponding differences in ptyalin concentration. But it is difficult always to obtain rates of secretion varying directly with the strength of acids used as stimulus. And the direct relation between the ptyalin concentration and the secretion rate is not a close one. A great difference in secretion rate is required in order to demonstrate the difference in diastatic power, but this is also true of the organic solids. Typical experiments illustrating this relation are given in Table I.

Despite the number of attempts we have so far been unable to demonstrate this relation between ptyalin concentration and secretion rate in the case of the rabbit's parotid saliva, probably because of the rapid

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[7] Ryan: This journal, 1909, xxiv, p. 234.

TABLE I.

SHOWING THE RELATION OF THE SECRETION RATE TO THE PTYALIN CONCENTRATION IN THE HUMAN PAROTID SALIVA. THE TESTS ARE MADE ON 25 C.C. 1 PER CENT SOLUTION OF BOILED STARCH + 0.1 C. C. PAROTID SALIVA, AT ROOM TEMPERATURE. ALL THE TESTS IN EACH GROUP ARE PARALLEL.

Stimulus.	Rate of secretion of 1 c.c. in min.	Rate of digestion in min.		
		Cleared.	Disappearance of starch.	Disappearance of erythrodextrin.
1. Acid (medium)	...	8	20	...
2. Acid (strong)	...	6	16	...
3. Acid (weak)	...	10	33	...
1. Acid (weak)	9	12	28	...
2. Acid (strong)	1	3	11	...
3. Acid (medium)	2.7	9	25	...
4. Acid (strong)	0.5	4	15	...
5. Acid (medium)	2.5	7	30	...
6. Acid (strong)	0.5	4	13	...
7. Acid (medium)	4.0	6	30	...
1. Sand	7	13	22	...
2. Acid	0.5	3	8	23
3. Sand	3.5	5	18	95
4. Acid	0.5	4	12	70
1. Sand	5	7	25	100
2. Acid	0.25	5	15	60
3. Sand	1.75	7	23	110
4. Acid	0.35	6	18	100

fatigue of the gland under experimental conditions. It is well known that the direct relation between secretion rate and organic solids is demonstrable only in the rested gland. Large monkeys provided with a permanent parotid fistula are necessary to establish the point beyond possible doubt, because in such animals the secretion rate can be controlled more accurately than in man.

While the experiments of Carlson and Ryan together with our results on man indicate quite strongly that the ptyalin in the saliva varies with the secretion rate in the same way as the rest of the organic solids, there is nothing in the literature on the other digestive glands, particularly the stomach and the pancreas, supporting these results. As a matter of fact, the work on the stomach and the pancreas seems to indicate that the ferment concentration of these glands varies quite independently of the secretion rate. These results on the salivary glands do not therefore permit any generalization. Possibly the apparent uselessness of ptyalin in the digestive processes is to be correlated with this difference between the laws governing the secretion of ptyalin and those governing the secretion of pepsin and the pancreatic ferments, the ptyalin concentration being governed by the same factors that determine the concentration of the other organic solids.

3. Qualitatively different stimuli in the mouth, such as acid, salt, sweet, bitter, mechanical, agreeable, disagreeable, yield no constant difference in the ptyalin concentration of the parotid saliva, unless they yield a constant and marked difference in the rate of secretion, as is the case, for example, with sand and acids. In man it is practically impossible, however, to secure uniform secretion rates in the case of the qualitatively different stimuli. Our results are therefore not conclusive on this point. But it is obvious that any investigation of the relation of ptyalin concentration or of the concentration of other substances in the saliva to different stimuli in the mouth or to different physiological states of the reflex centres must recognize the secretion rate factor.

THE ACTION OF EXTRACTS OF THE ANTERIOR LOBE OF THE PITUITARY GLAND UPON THE BLOOD PRESSURE.

BY WALTER W. HAMBURGER.

[*From the Hull Laboratory of Experimental Therapeutics, University of Chicago.*]

OLIVER and Schäfer[1] in 1895 showed that injections of pituitary extracts caused a rise in blood pressure. Howell[2] explained the apparently opposite results of Syzmanowicz[3] by isolating the two lobes and injecting each separately, whereupon the extracts made from the anterior lobe caused little if any change, while those from the posterior lobe caused an increase in blood pressure and slowing of the heart. Schäfer and Vincent[4] the following year found both a pressor and depressor substance in the posterior lobe. More recently von Cyon[5] investigated the depressor substance of the posterior lobe, which he calls "hypophysin." He found that it was soluble in alcohol and ether and that it caused an accelerated heart action. Von Cyon thinks its action similar to that of cholin.

Six years ago while investigating the action of intravenous injections of various glandular extracts I described a depressor effect produced by saline extracts of the anterior lobe.[6]

The conclusions reached at that time (1904) were as follows:

1. The intravenous injection of a saline extract of the hypophyseal (anterior) lobe of the pituitary body produces a distinct fall of blood pressure. This fall is accompanied usually by an acceleration and weakening of the heart.

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[1] OLIVER and SCHÄFER: Journal of physiology, 1895, xviii, p. 276.

[2] HOWELL: Journal of experimental medicine, 1898, iii, p. 245.

[3] SYZMANOWICZ: Archiv für die gesammte Physiologie, 1896, lxiv, p. 97.

[4] SCHÄFER and VINCENT: Journal of physiology, 1899, xxv, p. 87.

[5] VON CYON: Comptes rendus de l'Académie des Sciences, Paris, 1907, cxliv, p. 868.

[6] HAMBURGER: This journal, 1904, xi, p. 282.

2. A second injection of a saline extract of hypophyseal lobe immediately following the first, fails to produce any change in blood pressure. If a considerable interval be allowed to elapse, a second injection will produce a fall.

3. The active depressor substance is soluble in alcohol, glycerin, and salt solutions, but insoluble in ether. Repeated doses of the alcoholic extract, following each other immediately, are active.

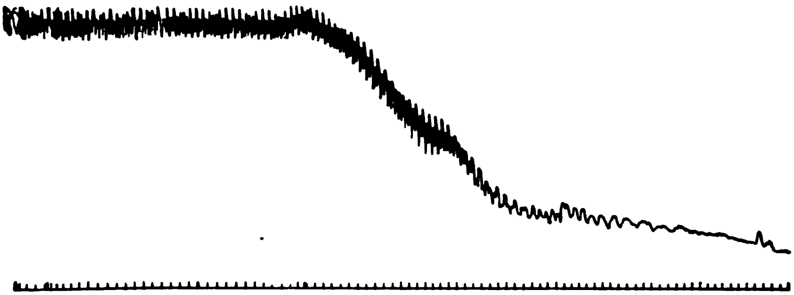

FIGURE 1. — One-half the original size. The injection of 10 c.c. of a saline extract of the anterior lobe of the pituitary gland causes a marked fall in the carotid blood pressure, recorded by a mercury manometer. The lower line records time in seconds and marks the atmospheric pressure. The dog died in shock.

4. A secondary rise above the normal follows the depressor effect produced by alcoholic extract of the hypophyseal lobe. It is also sometimes seen after injection of a saline extract. This may be due to an active pressor substance present in small amounts in the hypophyseal lobe or to the elimination of the inhibitory substance present in the saline extract.

Two years later, Schäfer and Herring[7] investigating the action of pituitary extracts upon the kidney were unable to obtain this depressor effect. My attention was called to this criticism through the kindness of Professor G. N. Stewart and it was decided to re-examine the problem. Until the present, this has been impossible, but I now have the results of a series of injections upon eight dogs. These findings, corroborative of the original experiments, form the basis for this short report.

The technique was identical with that used in 1904. Fresh material was obtained from the oxen of Schwarzchild and Sulzberger of the Union Stock Yards and prepared and injected on the same evening or the following morning. The fresh saline extracts prepared as a 25 per cent solution produced an immediate decided fall in blood pressure (Fig. 1).

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[7] SCHÄFER and HERRING: Philosophical transactions, 1906, B. 199, p. 1.

In a number of instances this fall in pressure was accompanied or succeeded by an apparently toxic action resulting in two cases in death of the animals from shock. Dogs 1 and 3, in spite of continued cardiac massage, artificial respiration, and intravenous injections of adrenalin chloride, died as an immediate result of the injections. This marked toxicity was not noted in the original experiments, probably because of the use of less concentrated suspensions. The fall in pressure, however, was constant and in accord with that obtained in the earlier work.

Schäfer and Herring describe their technique as follows: "The extract which we have used has been made up by taking one part by weight of the dry material (pituitary body), boiling it with 100 parts of Ringer's solution and filtering." And further, in a footnote they add, "to prevent putrefaction a little chloroform was always added to the bottle in which the fresh glands were collected, but this was always eliminated in the drying process." Feeling that these modifications in technique might explain the negative results of these investigators, it was decided to repeat their observations. Accordingly a little chloroform was added to a second portion of the fresh glands, the first portion of which had given an extract that lowered the blood pressure; the glands were then divided and spread out on glass plates and dried at room temperature, and this dried material was pulverized and boiled in 100 parts of Ringer's solution.[8] Ten cubic centimetres were injected into a freshly etherized dog. This caused no change whatsoever in blood pressure, rate and force of heart beat, respiration, or in the general condition of the animal. The conclusion seems justified that the pressure-lowering substance is destroyed as a result of some one or all of the steps in its preparation following the technique of Schäfer and Herring.

In conclusion I desire to thank Dr. S. A. Mathews for his courtesy in placing the laboratory at my disposal and for his assistance in the experiments.

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[8] It was found that 10 gm. of the fresh glands weighed approximately 1 gm. after drying at room temperature, so that the 25 per cent suspension of fresh glands used in these experiments is 2.5 times more concentrated than the 1 per cent solution of SCHÄFER and HERRING.

A QUANTITATIVE STUDY OF FARADIC STIMULATION.
IV. THE MAKE AND BREAK KEY.

By E. G. MARTIN.

[From the Laboratory of Physiology in the Harvard Medical School.]

FROM the beginning of the use of induction shocks for stimulating living tissues it has been recognized that the physiological intensities of these shocks are markedly affected by the manner of making or breaking the primary circuit. Helmholtz[1] called attention to this fact in his study of induced currents, and in the introductory paper of this series[2] the manipulation of the primary circuit was set down as one of the variable factors which must be controlled if faradic stimuli are to be made quantitatively useful.

SOURCES OF ERROR IN MAKE AND BREAK KEYS.

Errors affecting break shocks. — The method of measuring break induction shocks which I have developed in the course of this study[3] is based upon a discovery of Helmholtz, that the physiological effect of an induction shock depends, not upon its whole value, but upon its maximum intensity;[4] and that this maximum intensity is proportional to $\frac{M}{L}$; M being the mutual induction between primary and secondary coils, and L the self-induction of the secondary.

Now the relationship between the maximum intensity and $\frac{M}{L}$ depends upon the time required for the induced current to reach its maximum; for Helmholtz showed that the maximum intensity equals $\frac{M}{L}$ only when the time required for reaching it is zero, and falls below $\frac{M}{L}$ more and more as the time increases. It is clear, then, that the quantity $\frac{M}{L}$ cannot be used as a measure for break stimuli if the time required for the induced current to reach its maximum is subject to variation, but may be used so long as this time is constant, even though it may not be zero. Helmholtz showed that the induced current attains its maximum intensity at the instant the spark ceases to pass across the broken primary contact. The problem of obtaining uniform break shocks is, then, that of obtaining a key that shall give a uniform spark every time it is broken.

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[1] HELMHOLTZ: POGGENDORFF's Annalen der Physik und Chemie, 1851, lxxxiii, p. 505.

[2] MARTIN: This journal, 1908, xxii, p. 71.

[3] MARTIN: *Loc. cit.*, p. 116.

[4] HELMHOLTZ: *Loc. cit.*

The duration of the spark at a broken primary contact depends in part upon the intensity of the primary current, in part upon the amount of volatilization occurring at the contact, and in part upon the speed with which the points are separated. This last factor explains why keys operated by hand cannot be depended upon to give uniform results, and why some form of automatic key is required, since only thus can a uniform speed of separation be secured. Moreover, ordinary mercury keys cannot be depended on even when operated automatically, because of the tendency of mercury when not absolutely clean to cling in drops and thus vary the speed with which the contact points actually separate. In practically all keys there is some volatilization; platinum contacts giving the least, ordinary mercury contacts the most. It is impracticable to use always primary currents of a single intensity; but, as pointed out previously,[5] primary currents not exceeding 0.8 to 1 ampere intensity give sparks of virtually equal duration, other factors remaining constant.

Errors affecting make shocks. — The making of a primary circuit is not attended with sparking, so that the sources of error for makes are not the same as for breaks. In making a circuit we pass from a circuit of infinite resistance to one with the resistance of the closed circuit itself. It is during the passage from the first of these resistances to the second that the secondary current is induced. The more nearly instantaneous the change, the greater is the physiological intensity of the induced current. In hand-operated metal-contact keys there can be no assurance that the contact points will be pressed together with the same firmness twice in succession, so that to secure uniformity of contact automatic keys are required for make shocks as well as for breaks. A further and more serious defect in metal-contact keys for make shocks is their liability to rebound slightly, or to slip sidewise, thus giving not a single clean-cut make, but a succession of make, break, and make. So constantly has this defect shown itself in my experiments, even with carefully constructed automatic metal-contact keys, that I have found it necessary to use mercury contacts altogether in studying make shocks.

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[5] MARTIN: *Loc. cit.*, p. 71.

The considerations stated above lead to the following conclusions: that hand-operated keys are not to be depended on for uniform makes and breaks; that for break shocks platinum contacts are to be preferred to mercury because of their less volatilization, while for make shocks, on account of the rebound or side-slip of metal contacts, mercury affords the only trustworthy contact.

THE STEWART STOP-COCK KEY.

It is, of course, wholly undesirable to equip the primary circuit with two keys, — one of mercury to be used for making the circuit, and another of platinum for breaking it. I therefore set about the task of devising a single key which should serve satisfactorily the requirements of both make and break shocks. While thus engaged, I heard of a scheme suggested by Prof. C. C. Stewart which appeared likely to answer very well. Stewart's device was the simple one of filling a glass stop-cock with mercury, connecting the mercury on the two sides with the primary circuit, and opening and closing the circuit by turning the stop-cock. I accordingly fitted up a stop-cock with an arrangement for turning it automatically at the fall of a pendulum and tried it in the primary circuit. It gave very uniform results, both for break shocks and for make shocks; all my observations between November, 1906, and June, 1907, were made with this form of key. The chief objections to the stop-cock key were its bulkiness and complication when equipped with an automatic operating device, and the necessity of using a rather loose-fitting stop-cock because a tight-fitting one required lubrication to prevent binding, and the lubricant became quickly mixed with mercury, making it impossible to break the circuit by turning the cock. There was a certain amount of oxidation at the mercury surface, indicating that air had access to it; it was necessary, therefore, to renew the mercury in the stop-cock frequently.

THE VULCANITE KNIFE-BLADE KEY.

My experience with Stewart's stop-cock key suggested a key which would give even more uniform results than his, while being at the same time more compact and simpler in construction.

The principal defects in a mercury contact used for break shocks are, first, the tendency of the mercury particles to cling together and prolong the contact, a tendency which is much more marked in mercury contaminated with other metals or its own oxide than in perfectly clean mercury; and, second, the great volatilization occurring at the place where the contact is broken. For a break key of mercury to be efficient both these defects must be eliminated as completely as possible. How well these ends are secured in the key under consideration can best be judged from a description of the device itself.

The key consists of a block of vulcanite 30 mm. long, 20 mm. wide, and 25 mm. deep, having cut in it two vertical chambers (see Fig. 1), one (*a*) rectangular, 20 mm. long, 8 mm. wide, and 20 mm. deep; the other (*b*) cylindrical, 6 mm. in diameter and 20 mm. deep. A hole 3 mm. in diameter (*c*, Fig. 1) is bored through from one of these cavities to the other at a depth of about 16 mm. Each of the chambers is in electrical communication with a binding post, and when filled with mercury they are in electrical communication with each other through the connecting hole, *c*.

A strip of vulcanite, 18 mm. long, 8 mm. wide, and 1 mm. thick, flat on one side and on the other tapered toward the edges (*d*, Fig. 1), is supported at the top of the block by a horizontal rod working freely in a collar (*e*, Fig. 1), in such fashion as to press closely against the inner surface of the cavity, *a*, and when rotated about its axis of support to cover or uncover the opening *c*. When the vulcanite strip is brought over the opening, it cuts the mercury connection between cavities *a* and *b* and therefore breaks any electric circuit which may include them. It will be seen that this method of breaking a circuit has many points in its favor. The break cannot be delayed through the tendency of mercury drops to cling together, for the severance of the mercury column is not the withdrawal of one mass of mercury from another, but is the forcible interposition of a non-conductor in the path.[6] Moreover, the vulcanite strip cuts off not only the liquid mercury, but if it fits tightly, as it should, cuts off as well any mercury vapor that may be formed. Thus the effect of volatilization of mercury is minimized. Since the point where the break occurs is beneath a considerable depth of mercury air does not have access to it, and oxidation does not occur. I have found, as a matter of fact, that the same mercury may be used in one of these keys for months on end without any appreciable variation in the effectiveness of the break.

When the vulcanite strip is so rotated as to uncover the hole, *c*, the mercury in the two cavities reunites and thus makes the circuit. The reunion of the separated mercury masses should take place as smoothly as possible. To bring this about, the vulcanite knife blade is tapered at the edges so that it may plough through the mercury with as little disturbance as possible.

The operating device. — To secure uniformity of action it is necessary, as pointed out in a former paragraph, that the vulcanite blade be operated automatically, hand operation being liable to wide variations in the speed with which the contact is made or broken. The method adopted in this instrument is illustrated by the diagrams (Figs. 2 and 3). The axis of rotation of the blade (*o*, Figs. 1 and 2), after passing through the supporting collar (*e*, Fig. 1), is fastened into a triangular sheet of brass (*f*, Fig. 2), from whose apex project horizontally two brass arms, *w* and *w′*; these are bent at right angles at their outer ends, as shown in Fig. 3. From the tip of each of these arms a coiled spring (*L* and *L′*, Fig. 2) extends down to the end of a lever, *k* and *k′*. Each spring consists of twenty-seven turns of spring brass wire, 0.6 mm. in diameter. The length of the spring is about 16 mm., and the outside diameter of the coil 5 to 6 mm. The depression of either lever puts the spring connecting with it under tension and tends to draw downward the corresponding arm, rotating the vulcanite blade with it. To prevent movement of the blade until the spring has been put under a certain degree of tension two slits, *g* and *g′*, are cut into the lower edge of the triangle, *f*. A releasing device, *i*, is pressed upward against the lower edge of *f* by a stout spring, in such fashion that when either slit is engaged *f* is prevented from moving. Each of the levers, *k* and *k′*, bears at its tip an arm (*r*, *r'*, Fig. 3), which presses upon the release, and when the lever is depressed to a certain point disengages it, allowing the blade to rotate. The amount of motion of the blade is limited by setting two posts, *m* and *m'*, at such positions that the lower apices of *f* strike them when sufficient movement has occurred.

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[6] A device employing this same principle was described by Lombard in 1902, This journal, 1902, viii, p. xx.

After experimenting with various operating devices the one described above has been adopted as combining the greatest number of desirable features with the fewest defects. The two levers, *k* and *k'*, which are depressed alternately for making and breaking the circuit, are so placed as to lie naturally under the first and second fingers of either the right or the left hand. The springs, *l* and *l'*, need not be stiff, hence little pressure need be exerted upon the levers, and there is correspondingly little fatigue from continuous operation of the key. The springs are brought under tension only during the use of the instrument; when it is not in use, they hang free. Thus their stiffness does not vary with the lapse of time, as would be the case were they under constant tension.

FIGURE 1.

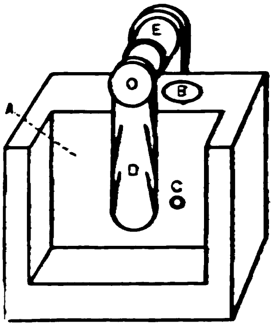

FIGURE 2.

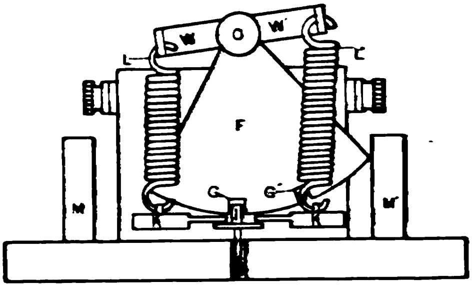

FIGURE 1. — Diagram illustrating the principle of the vulcanite knife-blade key. The front of the block is broken away to show the relations of the parts within the chamber *a*. *a* and *b*, mercury chambers; *c*, opening between *a* and *b*; *d*, vulcanite knife-blade supported upon axis *o*, which rotates within collar *e*.

FIGURE 2. — Diagram of the operating device for the knife-blade key; vertical view. *f*, triangle of brass bearing slits *g*, *g'*, and wings *w*, *w'*, rotating about axis *o*; *l*, *l'*, actuating springs; *k*, *k'*, levers for bringing tension upon springs, and at the same time operating release *i*; *m*, *m'*, stops for limiting motion of knife blade.

The short-circuiting device. — A desideratum in any key which is to be used for stimulating tissues with single induction shocks is a device for short-circuiting automatically either the make shocks or the break shocks at the will of the operator. The instrument under consideration lends itself so readily to the incorporation of such a device that I shall include a brief description of one, believing that the value of the key is enough enhanced thereby to justify its inclusion. The entire mechanism, shown in ground plan in Fig. 3, is mounted upon a slab of vulcanite, which in turn rests upon a base of soapstone, slate, or other suitable material. The vulcanite is cut away between and underneath the levers *k* and *k'*, as indicated at *s* (Fig. 3). A brass rod (*t*, Fig. 4) is mounted upon an axis, *u*, in such fashion that it can be rotated horizontally about this axis within the confines of the space *s*. At the end of the rod is a mercury cup, *v*. Two binding posts, *p* and *p'*, stand at one margin of the base. From *p* a wire leads through the body of the vulcanite block to the rod *t*, to which it is soldered near the axis of rotation of the rod. From *p'* two wires are carried through the block,

FIGURE 3.

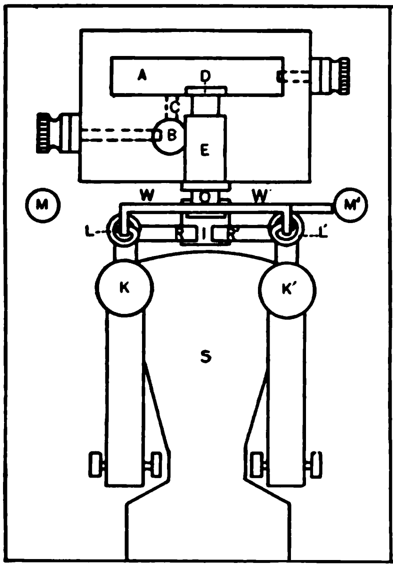

FIGURE 4.

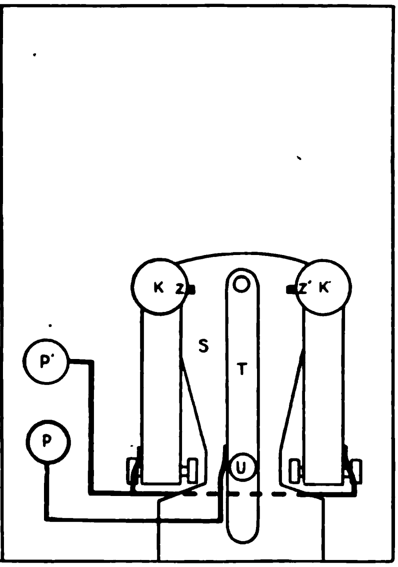

FIGURE 3. — Diagram of the operating device for the knife-blade key; horizontal view. Significance of letters the same as in Figs. 1 and 2. *s*, cavity in base for holding short-circuiting device.

FIGURE 4. — Diagram of the short-circuiting device. *t*, brass bar rotating horizontally about axis *u*, and bearing mercury cup *o*, which is in electrical communication with post *p*. *z*, *z'*, platinum pins mounted upon levers *k*, *k'*, and in electrical communication with post *p'*.

one to the axis of rotation of the lever *k*, to which it is soldered, the other to the axis of *k′*, where it is soldered likewise. Thus both levers are in electrical connection with the post *p′*, and the rod *t* in similar connection with the post *p*. Soldered to the levers *k* and *k′* at the points *z* and *z′* are pins of platinum projecting downward. These pins are so placed that the mercury cup *v* can be brought directly below one or the other of them according as *t* is rotated. Their length is so adjusted that the pin dips into the mercury when the lever is depressed enough to release the mechanism, but is clear of the mercury at all other times.

If the binding posts *p* and *p′* are connected in parallel into the secondary circuit of the inductorium and the rod *t* is rotated so as to bring the mercury cup below the lever which is pressed when the primary circuit is made, the left-hand one in this instrument, the make shocks are all short-circuited. Bringing the mercury cup below the other lever short-circuits all the break shocks. When the rod is placed in an intermediate position, neither makes nor breaks are affected. To prevent all possibility of accidental diversion of the secondary current into the hand of the operator, vulcanite shields are placed on the levers at the points where the fingers press upon them, and upon the handle by which the rod *t* is rotated.

DEMONSTRATION THAT THE KNIFE-BLADE KEY IS FREE FROM SERIOUS DEFECTS.

In previous paragraphs criticisms of ordinary make and break keys have been offered, and a description has been given of a key which in theory is largely free from the defects of other contact keys. It remains, however, to show by experiment that the knife-blade key is really better than other forms, or at least that it fulfils satisfactorily the requirements of quantitative work.

Since the only final test of the physiological efficiency of a stimulating mechanism is furnished by the responses of irritable tissue, and since there can be no assurance of a tissue preserving uniform irritability for more than a few hours at a time, it is obviously impossible to test the key by determining its efficiency from time to time over a long period and showing that it remains uniform. Some method of testing it must be devised which can be carried to completion in a single experiment. I believe that such a method is afforded by a comparison of the phys-

TABLE I.

DEMONSTRATION THAT MAKE SHOCKS AND BREAK SHOCKS OF EQUAL STIMULATING VALUE ARE OBTAINED WHEN THE PRIMARY CIRCUIT IS MADE AND BROKEN BY MEANS OF THE VULCANITE KNIFE-BLADE KEY.

Date.	Coil.	Break shocks.				Make shocks.					Per cent dif. between Z_b and Z_m.
		Sec. pos. cm.	$\frac{M}{L}$	Pri. cur. in amperes.	Z_b	Sec. pos. cm.	$\frac{M}{L}$	Pri. cur. in amperes.	Pri. voltage.	Z_m	
SERIES I.											
1909 Jan. 4	B	12	1100	0.0012	1.32	12	1100	0.0012	2	1.29	2.3
		16	250	0.0051	1.28	16	250	0.0053	2	1.21	5.5
		20	91	0.013	1.18	20	91	0.018	2	1.23	4.0
		24	46	0.026	1.20	24	46	0.052	2	1.24	3.2
Jan. 15	A	24	22	0.17	3.89[1]	24	22	0.61	2	3.90	0.25
Jan. 16		28	12.8	0.12	1.58[1]	18	69	0.024	4	1.58	0.0
		28	12.8	0.132	1.74[1]	18	69	0.026	8	1.75	0.58
Jan. 18		30	10	0.093	0.93	18	69	0.015	2	0.975	4.6
		20	43.5	0.13	5.84[1]	16	120	0.0605	2	5.86	0.34
Jan. 19		26	16.8	0.12	2.07[1]	12	600	0.0034	2	2.04	1.45
SERIES 2.											
1910 Jan. 4	B	16	250	0.024	6.0	16	250	0.030	4	5.96	0.67
		24	46	0.12	5.66	24	46	0.32	8	6.0	5.7
Jan. 5		20	91	0.0445	4.05	20	91	0.059	6	3.96	2.2
Jan. 6		12	1100	0.0106	11.7	12	1100	0.0138	2	12.2	4.0
		14	530	0.023	12.2	14	530	0.0295	4	12.3	0.8
		16	250	0.049	12.2	16	250	0.069	6	12.2	0.0
		18	145	0.084	12.2	18	145	0.13	8	11.9	2.5
		20	91	0.12	11.2	20	91	0.22	10	11.2	0.0

[1] The correction factor for core magnetization is introduced. See This journal, 1908, xxii, p. 122.

iological effects of make shocks and break shocks upon the same irritable tissue. Since the defects of a key by which the two sorts of shocks are affected are not the same (see p. 181), and since, furthermore, the physiological effects of break shocks are computed according to one formula and those of make shocks according to another,[7] it seems to me wholly unlikely that corresponding values for make and break stimuli could be secured with a key which is at all markedly defective. I submit, therefore, as a criterion for any make and break key which is to be used in quantitative work that make and break stimuli generated through its agency must agree in value within a moderate limit of error, perhaps 5 or 6 per cent at the outside.

In Table I are presented the results of two series of tests of a knife-blade key separated by an interval of one year. To make the tests thorough, the conditions were varied rather widely within each series. The results seem to show that the particular key tested is reliable, so far as can be judged from this criterion.

As a further check upon the reliability of the knife-blade key, two were connected in series in the primary circuit of an inductorium, and their efficiency was directly compared. Both keys were made in the machine shop connected with this laboratory, but not at the same time nor by the same mechanic. Both were made according to the design presented in previous paragraphs; they differed, however, in at least one important detail, namely, the stiffness of the springs (*l* and *l′*, Fig. 2) which actuate the knife-blade mechanism. By direct measurement the tension of each spring of one key at the instant when the catch was released was shown to be about 130 gm., of the other key nearly 200 gm. No particular pains were taken to have the depths of mercury the same in the chambers of the two keys. There was, in fact, no attempt to have them identical in every respect nor more nearly alike than any two keys made from a single design would be apt to be. The result of the experiment was that no difference between the two keys could be observed, so far as they affected the intensity of primary current required to bring about a faradic shock of given stimulating value. Tests were made of both make and break stimuli; with the secondary coil at positions ranging from 0 cm. to 24 cm.; and with primary currents varying from 0.0013 ampere to 0.2 ampere.

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[7] MARTIN: This journal, 1909, xxiv, p. 278.

THE EFFECTS OF DISTILLED WATER AND OF VARIOUS SOLUTIONS ON THE WEIGHT AND LENGTH OF STRIATED MUSCLE.

BY EDWARD B. MEIGS.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

INTRODUCTION.

FOR a number of years there has been a gradual accumulation of evidence pointing to two important conclusions in regard to the contraction of striated muscle. The first is that contraction is the direct mechanical result of the swelling of the fibrillæ or sarcostyles at the expense of the sarcoplasmic fluid; the second, that the maintained contraction which is characteristic of the condition known as rigor is caused by an accumulation of lactic acid within the muscle. These conclusions, far from being mutually exclusive, agree very well with one another. It has been known for a long time that the sarcostyles of at least one form of striated muscle show a marked tendency to swell in even very dilute acid solutions.[1] If, therefore, it can be shown that a muscle going into rigor always produces acid, and that swelling of the muscle sarcostyles causes them to shorten, a complete, though rough, explanation of the shortening which accompanies rigor seems to have been given.[2]

Striated muscle may be artificially caused to swell or to lose water by various reagents, and experiments with such reagents ought to throw light on the question whether or not contraction really is a matter of swelling of the sarcostyles. It must be remembered, however, that the muscle as a whole might absorb or lose water without the sarcostyles taking any part in the process, for it is perfectly conceivable that the additional fluid might be held either in the spaces between the fibres or in the sarcoplasmic spaces. Further, it has long been clear that the taking up and loss of water by muscle immersed in various solutions of acids, bases, and salts or in distilled water is a complicated process; and this field must be more or less cleared up before any safe conclusions can be drawn from the kind of experiments proposed. The question of the osmotic properties of the various structures of which a striated muscle is composed is, of course, in itself a very important one.

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[1] MCDOUGALL: The journal of anatomy and physiology, 1898, xxxii, p. 193; SCHÄFER: Monthly international journal of anatomy and physiology, 1891, viii, p. 203.

[2] Reviews of parts of this subject and references to its literature are to be found in the Ergebnisse der Physiologie, 1909, viii, pp. 147 *et seq.*, and The journal of physiology, 1909, xxxix, p. 385.

It will be convenient to divide this article into two parts, in the first of which I shall consider the changes of weight undergone by striated muscle in distilled water and various solutions of electrolytes and non-electrolytes; I shall endeavor to show that the swelling of striated muscle in distilled water is a complex of two quite distinct processes. In the second part I shall consider the relation between the changes of weight described in the first part and the changes of length which may be shown to accompany them.

PART I. THE CHANGES OF WEIGHT UNDERGONE BY STRIATED MUSCLE IN DISTILLED WATER AND IN VARIOUS SOLUTIONS.

The changes produced in muscle by distilled water. — The complicated nature of the changes produced in muscle by distilled water is well seen if one follows the changes in weight undergone by frog's muscle immersed for from twenty-four to forty-eight hours in this fluid. Fischer has studied these phenomena in the hind leg of the tree frog and states that the muscle first gains in weight, then loses, and then gains again.[3] This series of changes, he says, is very characteristic for muscle immersed in distilled water.

I have repeated Fischer's experiment with the frog's sartorius and obtained essentially the same results. The sartorius is, of course, a much smaller mass of muscle than that used by Fischer, and therefore all the periods of swelling and loss of water were shorter in my experiment. I also obtained a second period of very slow loss in weight, which was no doubt absent from Fischer's curves because he did not carry them far enough. The curve in Fig. 1 represents the changes in weight of a sartorius immersed in distilled water in one of my typical experiments.

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[3] FISCHER: Archiv für die gesammte Physiologie, 1908, cxxiv, p. 74.

It has been desirable for my purposes to have the time relations of all my experiments readily comparable, and I have therefore used the sartorius in almost all cases. In all the experiments to be subsequently described, therefore, it may be assumed that the frog's sartorius has been used unless the contrary has been specifically stated. The water which I used was distilled over glass, and my solutions were made with this and chemically pure substances. The muscles in which the changes in weight were followed were dried in as uniform a manner as possible on filter paper and weighed on an analytical balance. The limits of error are certainly within 2 milligrams. It will be noticed that two of the weight curves, namely, those in which the attempt was made to follow the changes in weight of muscle immersed in fluids at low temperatures, are quite irregular (see Figs. 4 and 5). The muscles used in these experiments were taken out of the cold fluids and weighed at room temperature. It cannot therefore be considered that these curves accurately represent the course of swelling at the temperature given, but they are sufficient to indicate in a rough way the effects of temperature.

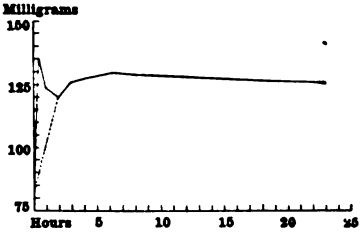

FIGURE 1. — Changes in weight undergone by a sartorius immersed in distilled water of which the temperature varied between 19° and 20°. (The dotted line represents the hypothetical early swelling of the sarcostyles.)

A great deal of work has been done on the changes which occur in muscle immersed in distilled water and in various solutions of electrolytes and non-electrolytes, and a full knowledge of the facts already recorded helps very much toward an understanding of the structure and many of the properties of muscle. The field is a complicated one, and I shall be obliged to take up the new and old observations in the order which seems most advantageous for exposition.

In the first place, then, a muscle immersed in distilled water undergoes chemical as well as physical changes. Thirty-three years ago Du Bois-Reymond reported that a frog's muscle placed in distilled water becomes acid in an hour.[4] In a recent paper [5] I have added some more exact observations to those of Du Bois-Reymond and have discussed some apparently contrary results which had been reported.

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[4] DU BOIS-REYMOND: Muskel und Nervenphysik, Leipzig, 1877, Bd. ii, p. 17.

[5] MEIGS: The journal of physiology, 1909, xxxix, p. 385.

Since the publication of this paper I have made still further observations with the view of determining roughly how soon a frog's sartorius becomes acid and loses its irritability in distilled water, and whether irritability may be restored after it has once been lost.

It is sometimes possible to show that five minutes' immersion in distilled water at 20° renders a sartorius slightly more acid to litmus than fresh muscle. It takes somewhat longer, however, to destroy completely the irritability of all the fibres; a small number of fibres at the thick upper end of the muscle may remain irritable for even twenty-five minutes in distilled water at 18°. It seems at first sight remarkable that the acid reaction is demonstrable before the complete loss of irritability, but this relation is easy to understand if one considers that the distilled water does not affect all the fibres at the same time. The outer fibres are rendered acid and unirritable within five minutes, while the inner fibres retain their irritability and probably their normal reaction somewhat longer. The contractions of the still irritable inner fibres are, of course, still visible even after they are completely surrounded by a surface layer of acid and unirritable fibres.

To determine the question whether the irritability of any given portion of the muscle may be restored after it has once been lost, I have kept sartorii for varying periods in distilled water and then transferred them to Ringer's solution and tested them at intervals. Ringer's solution was used because it is known that muscles immersed in this fluid retain their irritability for a long time, and it has been shown that transferring a muscle to Ringer's solution will cause it to assume its original length and reaction after it has been for a considerable time in distilled water.[6] It will be shown in this article (p. 196) that salt solutions tend to remove the swelling caused by distilled water.

In one case a sartorius was kept for twenty minutes in distilled water at 18°, and then for twenty-four hours in Ringer's solution at between 15° and 18°. It was completely unirritable at the end of the first twenty minutes, and remained so during the whole twenty-four hours' immersion in Ringer's solution. In another case a sartorius appeared to be completely unirritable after twenty-five minutes' immersion in distilled water at 18°. After it had been an hour in Ringer's solution at 18°, however, it was possible to show that a few fibres at the thick upper end were slightly irritable, though all the rest of the muscle was completely unirritable. The tissue was left in Ringer's solution at between 15° and 18° for twenty-three hours more, and the condition described persisted through the whole period. This experiment points even more conclusively than the preceding one to the probability that the irritability of any given portion of the muscle cannot be restored after it has once been destroyed by distilled water. After the twenty-five minutes in distilled water the outer fibres of the muscle were much shortened, and their shortening would mask any contraction of the still irritable inner fibres. The Ringer solution, however, removed the contraction of the outer fibres without restoring their irritability, and the contraction of the inner fibres then became visible. The fact that the slight irritability of the few fibres and the complete unirritability of the great majority persisted through the whole twenty-three hours' immersion in Ringer's solution is strong evidence for the view that the irritability of a frog's muscle fibre, once destroyed by distilled water, cannot be restored by Ringer's solution.

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[6] MEIGS: *Loc. cit.*, pp. 387–389.

It is clear, then, that a frog's sartorius placed in distilled water at from 15° to 20° becomes rapidly acid and loses its irritability irretrievably in about twenty minutes. I have dwelt at some length on the time at which the irretrievable loss of irritability occurs, because this loss is, as nearly as can be judged, synchronous with the beginning of the first loss of weight which occurs in a muscle immersed in distilled water. Fig. 2 represents in some detail the curve of change in weight during the early stages of water rigor. It will be seen that the muscle begins to lose weight after about twenty minutes. Overton has demonstrated that the living muscle fibres are surrounded by osmotic membranes permeable to water and to many organic substances, but impermeable to sugars and inorganic salts; these membranes are easily destroyed by the action of acids.[7] That the destruction of the osmotic membranes surrounding the fibres would deprive the muscles of irritability is clear from the work of Overton. He has shown that the maintenance of this property depends on the existence of the semi-permeable membrane surrounding the fibre with the muscle salts on one side and the blood salts (or, at any rate, a considerable proportion of NaCl) on the other.[8] It is probable, therefore, that both the first loss in weight and the loss of irritability are due to the destruction of the semi-permeable properties of Overton's membrane.

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[7] Overton: Archiv für die gesammte Physiologie, xcii, pp. 115 and 356; also, 1904, cv, p. 207.

[8] Overton: *Loc. cit.*, 1902, xcii, p. 346.

Distinction between the swelling of living and dead muscle. — Overton recognized quite clearly that the semi-permeable membranes surrounding the fibres exist only in living muscle, and that dead muscle may be made to imbibe fluid by a process which is possibly quite different from what is ordinarily known as osmosis. He speaks of the swelling which muscle may undergo in strong salt solutions, and says that this swelling may be due to the formation of acid and consequent increased tendency toward imbibition ("Quellbarkeit") in some part of the muscle.[9] Fischer[10] has studied in some detail the swelling of muscle in various solutions of acids, bases, salts, and sugars. He does not consider the question whether his tissue was living or dead, and a careful study of his observations shows that the latter alternative must always have been the case long before the end of the experiment, and usually very early in it. His results do not at all conflict with those of Overton which demonstrate the semi-permeable membranes surrounding the living fibres; Fischer's results show simply that dead muscle, like solutions of fibrin, gelatin, and other colloids, takes up water in acid and alkaline solutions and tends to lose it in solutions of neutral salts.

The differences between the osmotic properties of living and dead muscle may be strikingly demonstrated in the following manner. A frog's sartorius is placed for about half an hour in distilled water (it may be supposed that the temperature in this and all the succeeding manipulations is maintained at 20°). By the end of this time it is much swollen, shortened, and acid; the irritability is entirely destroyed, and the osmotic membranes surrounding the fibres are broken down. This muscle is now immersed for several hours in Ringer's solution, at the end of which time it will have regained its original weight, length, and reaction, though it will still be completely unirritable to electric stimuli. Such a muscle is, however, still capable of gaining in weight and shortening when immersed in distilled water.

But the curve of gain in weight of a muscle treated in this way is quite different from that which is obtained when a fresh muscle is placed in distilled water. Fig. 2 shows the manner in which a fresh muscle takes up distilled water; Fig. 3, that characteristic for a muscle which has been treated in the manner described above. It will be seen that the curve of Fig. 2 is exponential in character; the fresh muscle takes up most water in the first five minutes, less in the next five minutes, and so on. The dead muscle, on the other hand, takes up water more and more rapidly through the first fifteen or twenty minutes of its immersion.

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[9] Overton: *Loc. cit.*, pp. 157 and 158.

[10] Fischer: This journal, 1907, xx, p. 330; and Archiv für die gesammte Physiologie, 1908, cxxiv, p. 69.

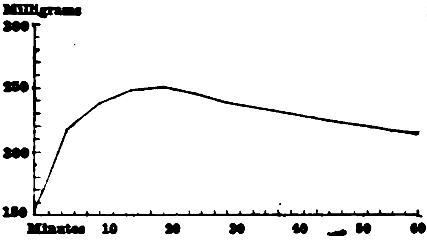

Figure 2.— Changes in weight undergone by a sartorius immersed in distilled water of which the temperature varied between 19° and 20°.

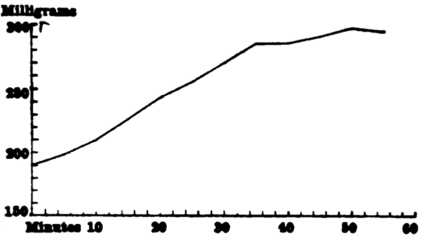

Figure 3.— Changes in weight undergone by a sartorius immersed in distilled water of which the temperature varied between 17° and 17.5°. The osmotic membranes of this muscle had been destroyed.

Another interesting difference in the manner of swelling of living and dead muscle is brought out by studying the effects of temperature on the two curves. The results are given in Figs. 4 and 5. It will be seen that the fresh muscle takes up water less rapidly at 0° than at 20° only in the first five minutes, and that in all the succeeding periods the intake is more rapid at the lower temperature. The dead muscle, on

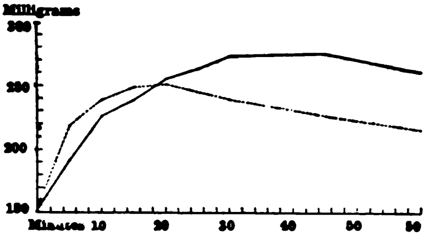

Figure 4.— Changes in weight undergone by a sartorius immersed in distilled water at 0°. For comparison the curve of Fig. 2. is drawn in as a dotted line (see p. 193).

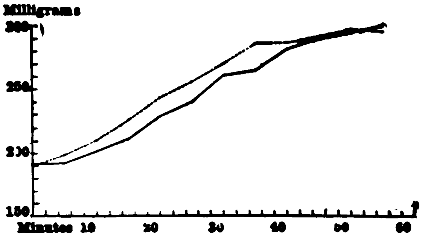

Figure 5.— Changes in weight undergone by a sartorius immersed in distilled water at 0°. (The osmotic membranes of this muscle had been destroyed.) For comparison the curve of Fig. 3 is drawn in as a dotted line (see p. 193).

the other hand, takes up water more slowly at the lower temperature through the first twenty-five minutes of immersion. The effects of temperature on the swelling of fresh muscle in distilled water are easily explained on the hypothesis that this swelling is dependent on the presence of semi-permeable membranes surrounding the fibres, provided that certain easily demonstrated facts are kept in mind. It has been shown already that a muscle placed in distilled water produces acid, and it is very easy to show, what might almost be taken for granted, that the acid production is more rapid at higher temperatures.

To show this fellow sartorii were kept, the one for fifteen minutes in distilled water at 22°, the other for half an hour in distilled water at 0°. At the ends of these periods the reaction of the first was decidedly more acid than that of the second. In another case the temperatures were 18° and 0°, and the times fifteen and forty-five minutes respectively. The reactions of the two muscles used in this experiment were indistinguishable from one another.

If these facts are remembered in connection with the fact that acid destroys the semi-permeability of Overton's membranes, the effect of temperature on the early swelling of muscle in distilled water may be explained as follows. The muscle swells more rapidly during the first five minutes at the higher temperature because the acid is formed more rapidly at that temperature and acts at first with the salts to cause a more rapid diffusion of water into the fibres. By the end of the first five minutes, however, the acid has already begun to destroy the semi-permeable membranes of the outer fibres; these begin to lose fluid, and their loss of fluid renders slower the gain in weight of the muscle as a whole. At 0°, on the other hand, the production of acid and consequent destruction of semi-permeable membranes is very much slower, while the rapidity of the physical diffusion of water into the muscle fibres is comparatively little affected. As a consequence, all the fibres swell rapidly from the fifth minute onward, and they all reach their fullest distention much more nearly at the same time than is the case at the higher temperature.

In the dead muscle, the process by which water is absorbed is probably entirely different. As the salts diffuse out of the tissue, it is rendered more and more capable of absorbing water. During the first fi[illegible] minutes it is improbable that the salt content of any but the outer layer of tissue is much altered; and only a small proportion of the muscle, therefore, takes part in the process of imbibition. But, as time goes on, the salt content of more and more of the tissue becomes lowered, and the process of imbibition in the muscle as a whole goes on faster and faster until a certain proportion of it becomes saturated. The low temperature, no doubt, slows the whole process by decreasing the rate at which the salts diffuse out of the tissue.

The swellings of living and of dead muscle may be most sharply distinguished from one another by comparing their behavior in solutions of cane sugar slightly hypertonic to 0.7 per cent NaCl solution. Living muscle maintains its original weight for a long time in such solutions, as Overton has shown, while muscle, which has been treated first with distilled water and then with Ringer's solution, swells quite rapidly. Fig. 6 shows the changes in weight undergone by fellow sartorii immersed in 7.5 per cent cane sugar solution. One of these muscles was immersed while still living in the sugar solution; the other was first treated with distilled water and Ringer's solution as described in the legend under the figure.

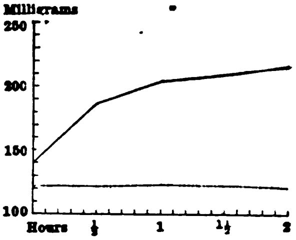

FIGURE 6.— Changes in weight undergone by a living sartorius (broken line) and a dead sartorius (unbroken line) immersed in 7.5% (slightly hypertonic) cane sugar solution at 20°. The dead muscle had been treated an hour with distilled water, and then for two hours with Ringer's solution at 21°.

Fischer's main results are that dead muscle tends to swell in distilled water and in weak acids and alkalies and to lose water in solutions of neutral salts. The following experiments may serve as a rough confirmation of these results:

	Absolute weight. gm.	Percentage change.
A frog's sartorius weighed fresh	0.075	. . .
After twenty-three hours in distilled water . . .	0.128	+71
After one half hour more in 0.7 per cent NaCl sol.	0.101	−21
A second sartorius weighed fresh	0.082	. . .
After twenty-three hours in distilled water . . .	0.124	+51
After one half hour more in 0.4 per cent lactic acid	0.196	+59
A third sartorius weighed fresh	0.094	. . .
After twenty-three hours in distilled water . . .	0.135	+42
After one half hour more in 0.2 per cent NaOH sol.	0.167	+24

The temperature of the fluids used in all three experiments varied between 19° and 21°.

In 5 per cent or stronger NaCl solutions dead muscle tends to gain in weight, and more than can be explained by supposing that the gain is due merely to the diffusion of salt into the muscle. The following experiments show the amount of gain which takes place under these circumstances:

	Absolute weight. gm.	Percentage change.
A sartorius after eighteen hours in distilled water weighed	0.250	...
After two hours more in 5 per cent NaCl sol.	0.270	+8
A semitendinosus after four hours in distilled water weighed	0.243	...
After five hours more in 5 per cent NaCl sol.	0.277	+14
A second semitendinosus after four hours in distilled water weighed	0.191	...
After five hours more in 10 per cent NaCl sol.	0.229	+20

The temperature in these experiments varied between 20° and 21°

It is evident, of course, that the diffusion of salt into a muscle from 5 per cent solution could account for a gain in weight of only somethin less than 5 per cent, unless there was some accompanying increase i volume. In the same way a muscle could not gain more than 10 p cent in weight from a 10 per cent salt solution, unless there was an a companying increase in volume. But in the experiments given t muscles gain respectively 8 per cent and 14 per cent in the 5 per c solutions, and 20 per cent in the 10 per cent solution. It m be therefore that these strong salt solutions produce a tendency ward actual increase in volume; this result is no doubt analog to certain observations on blood serum which Moore and Roaf[11] h reported.

All these facts show that the muscle fibre is capable of und two distinct kinds of swelling. The first depends on th the peculiar semi-permeable membrane which Over strated; this kind of swelling has many of the ch

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[11] Moore and Roaf: Biochemical journal, 19

Effects of Distilled Water and of [illegible] Solution

[illegible]

and can impress their form on a mould indicates that the sarcoplasm must flow away during these manipulations.

It follows from these considerations that the second gain in weight of muscle in distilled water (see Fig. 1) is purely the result of the swelling of the sarcostyles. For the beginning of the first loss of weight must mean the destruction of the osmotic properties of Overton's membranes; and after this happened, the fluid sarcoplasm could take no further part in the swelling of the muscle, unless it be supposed that the membranes are built up again. But this supposition is impossible, for it has been shown (see pp. 196, 199 and Figs. 3, 4, 5, and 6) that the swelling of muscle after the destruction of Overton's membranes is of such a character as to preclude the possibility that it has anything to do with the existence of such membranes.

Summary of the changes produced by distilled water. — The following description of the processes which occur in a muscle immersed in distilled water will serve as a summary of the observations so far reported.

The fibres of a sartorius immersed in distilled water at 20° C. take up water rapidly by a purely physical process for a period of about twenty minutes. The consequent dilution of the fibre contents results in a production of lactic acid, which begins in less than five minutes after the beginning of the experiment. The presence of the lactic acid causes in the first place a tendency for the sarcostyles to absorb fluid; in the second, it destroys the irritability of the muscle and the osmotic properties of the membranes surrounding the fibres. The destruction of these osmotic membranes results in a passage of fluid from the sarcoplasmic spaces to the exterior; the absorption of fluid by the sarcostyles, however, is not affected by this and continues through the whole course of it, so that for a period of about two hours fluid is passing from the sarcoplasmic spaces to the sarcostyles on the one hand and to the exterior on the other. At the end of about two hours there is no longer any tendency for fluid to pass from the sarcoplasmic spaces to the exterior; the sarcostyles, however, go on absorbing fluid, the muscle as a whole begins to gain in weight, and continues to do so for from two to four hours more. During all this time lactic acid has been escaping fairly rapidly from the muscle to the surrounding fluid. I have made sure of this point by applying the thiophene test for lactic acid[18] to bodies of distilled water in which muscles have been kept for various periods. It is easy to demonstrate considerable quantities of lactic acid in water in which muscle has been kept for only an hour. No doubt the production of lactic acid by the muscle becomes gradually slower and finally stops; but before the final stoppage a point must be reached where the acid escapes to the surroundings faster than it is formed. As soon as this happens the acid reaction of the muscle begins to be reduced and with it the power of the sarcostyles to absorb and hold water. This probably accounts for the very slow loss of weight by the muscle from the fifth or sixth hour onward.

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[18] FLETCHER and HOPKINS: The journal of physiology, 1907, xxxv, p. 308.

PART II. THE CHANGES IN LENGTH WHICH ACCOMPANY THE CHANGES IN WEIGHT DESCRIBED IN PART I.

Existing evidence for the view that dead sarcostyles may be made to shorten by increasing their volume. — If the above account of the changes which take place in a muscle immersed in distilled water be correct, the tissue, after a considerable period of immersion, is an interesting object for study. The osmotic membranes of the fibres have been destroyed, the superfluous fluid has escaped from the sarcoplasmic spaces, the power of producing lactic acid has probably been lost, and the tissue (it is perhaps hardly justifiable to call it any longer a muscle) is to all intents and purposes a bundle of dead sarcostyles, which may be caused to swell by certain artificial means and to lose water by certain others.

McDougall's theory of muscular contraction supposes that the sarcostyles of living muscle are so constructed that any increase in their volume tends to cause them to shorten; and he has given some evidence to show that the sarcostyles of insects' wing muscles retain this property after they have been killed by certain fixing reagents.[19] This evidence, however, depends on the making of rather difficult microscopic observations, and would be much strengthened by support gained from observations on other kinds of muscle.

Hürthle, though he is on the whole hostile to McDougall's theory, has furnished some evidence of this sort. He finds that the fibres of the leg muscles of insects may be dried in a sulphuric acid desiccator at about −15° C., and that if they are then immersed in glycerine they assume an appearance closely resembling that of the uncontracted living fibres. If, however, such fibres are placed in water or Ringer solution, they swell more or less and at the same time shorten to about half their previous length and undergo all the microscopic changes which occur in the living contracting fibres.[20] Hürthle admits that these observations show that his dried fibres have the mechanical structure attributed by McDougall to the living muscle, but he seems to think that this structure is either acquired during the process of drying or else is present by chance in the living muscle and not used in the normal process of contraction.

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[19] McDOUGALL: The journal of anatomy and physiology, 1897, xxxi, p. 430.

To my mind both these suppositions are in the highest degree improbable. It requires some ingenuity to construct a machine which will shorten at all when the volume of its contents is increased, and to produce a machine which will shorten to less than two thirds of its original length under such circumstances is a mechanical problem of extreme difficulty. If the muscle has such a structure, it is hardly conceivable that it is there by chance or grows while the tissue is being dried at −15° C. And why should the dried muscle "undergo all the microscopic changes characteristic of normal contraction," if its shortening has no relation to that which occurs under physiological circumstances?

Detailed evidence for McDougall's theory from frog's muscle. — The study of frog's muscle treated with distilled water furnishes evidence for the view that this form of muscle also has the structure attributed by McDougall to striated muscle, and that this structure persists to a certain extent after what is commonly called the death of the muscle. It is to the presentation of this evidence that the succeeding paragraphs are to be devoted.

In experimenting on the changes of length undergone by frog's muscle immersed in distilled water and in various solutions of electrolytes and non-electrolytes I have uniformly used the sartorius. The muscle was arranged to record on a slow kymograph while immersed in the solutions, and was in all cases under a tension of 1 gm. The temperature was usually in the neighborhood of 20°. I have not thought it worth while to publish reproductions of the original records, because these are often of an inconvenient shape, and the details of the curves vary so much under even the same experimental conditions that no single example can be considered typical. The chief data obtained from the records have been transferred to diagrams, which give the general outlines of the curves with sufficient accuracy.

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[20] HÜRTHLE: Archiv für die gesammte Physiologie, 1909, cxxvi, pp. 125–130.

Fig. 7 shows the changes in length of a muscle immersed for eleven hours in distilled water at 20°. It will be seen that the muscle shortens at a gradually decreasing rate for some four hours and then lengthens very slowly for the next seven. If Fig. 7 be compared with Fig. 1, which gives the weight changes under similar circumstances, it will be seen that the curve of length change corresponds fairly well with that portion of the weight-change curve which may be supposed to represent the course of the swelling of the sarcostyles (the dotted line in Fig. 1 represents the hypothetical early swelling of the sarcostyles which is masked by changes in the amount of fluid contained in the sarcoplasmic spaces). The fact that the length-change curve reaches its highest point two hours earlier than the other may be due to individual variation, but it has more probably another explanation which will be considered later (see pp. 209 and 210).

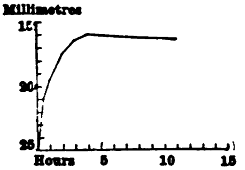

FIGURE 7.— Changes in length undergone by a sartorius immersed in distilled water at 20°.

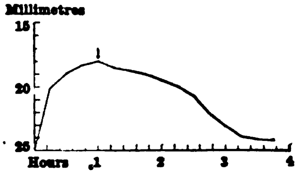

FIGURE 8.— Changes in length undergone by a sartorius immersed first in distilled water and then (arrow) in 0.7 per cent NaCl solution of which the temperature varied between 21.5° and 22°.

A muscle in water rigor may be made to lengthen, by substituting 0.7 per cent NaCl solution for the distilled water. I have tried this substitution at various stages up to twelve hours and have found that the contraction may always be made to disappear entirely. Fig. 8 represents the effects of applying 0.7 per cent NaCl solution at an earlier stage of rigor, and Fig. 9, the same thing at a later stage. It is highly probable that the muscle retains the power of lengthening under the influence of 0.7 per cent NaCl solution indefinitely, but I have not thought it worth while to carry the experiments beyond twelve hours.

The water-rigor contraction, at least in its early stages, is more rapidly removed by a 0.7 per cent solution which contains a small amount of $NaHCO_3$ than by a pure 0.7 per cent NaCl solution. Figs. 8 and 10 are curves of water rigor and its removal made from companion muscles under similar conditions of temperature, weighting, etc. In Fig. 8 the distilled water was replaced after one hour by pure 0.7 per cent NaCl solution, and in Fig. 10 by 0.7 per cent NaCl solution to which 0.03 per cent of $NaHCO_3$ had been added.

It may be shown that 0.7 per cent NaCl solution to which 0.03 per cent $NaHCO_3$ has been added removes the acid reaction of a muscle in water rigor, while pure 0.7 per cent NaCl solution has little or no tendency to do so.

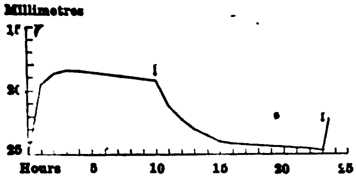

FIGURE 9.— Changes in the length of a sartorius immersed first in distilled water, then in 0.7 per cent NaCl solution (first arrow) and finally in 0.4 per cent lactic acid (second arrow). The temperature varied between 20° and 22°.

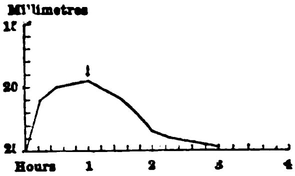

FIGURE 10.— Changes in length undergone by a sartorius immersed first in distilled water and then in a mixture containing 0.7 per cent NaCl and 0.03 per cent $NaHCO_3$ (arrow). The temperature varied between 21.5° and 22°.

To show this three sartorii of as nearly as possible the same size were selected and placed in distilled water at 20°. At the end of an hour sartorius 1 was removed from the distilled water and placed in the above-described mixture of NaCl and $NaHCO_3$; sartorius 2 was placed in pure 0.7 per cent NaCl solution; and sartorius 3 was left in distilled water. After two hours more the reactions of the three muscles to litmus were compared with each other. Sartorii 2 and 3 were strongly acid and indistinguishable, from one another in reaction; sartorius 1 had the same reaction as a fresh muscle.

There is little doubt that the $NaHCO_3$ removes the acid reaction of muscles in water rigor by reacting with the lactic acid to form sodium lactate, water, and CO_2. It is not, however, simply a matter of the muscle's assuming the reaction of the solution in which it is immersed. A fresh muscle, though very nearly neutral in the physico-chemical sense, turns purple litmus decidedly toward the blue, and the same thing is true of a muscle which has been thrown into water rigor and then immersed for some hours in a solution containing 0.7 per cent NaCl and 0.03 per cent $NaHCO_3$. The solution itself, however, has no effect on the color of purple litmus.

The removal of the water rigor contraction by 0.7 per cent NaCl solution and more rapidly by the mixture of NaCl and $NaHCO_3$ is readily explained on the hypothesis that the contraction in question is the direct mechanical result of the swelling of the sarcostyles. It was shown in the first part of this article that muscle, which has been caused to swell by several hours' immersion in distilled water loses fluid when transferred to 0.7 per cent NaCl solution (see p. 199). If shortening is the result of swelling of the sarcostyles, then lengthening may be expected as the result of any process which tends to reduce that swelling. It was shown also in the first part (p. 199) that an acid reaction tends to produce swelling, and it is easy to see, therefore, why $NaHCO_3$, which removes the acid reaction, should hasten the lengthening.

Solutions which contain large quantities of $NaHCO_3$ (as, for instance, a solution containing 0.35 per cent NaCl and 0.5 per cent $NaHCO_3$) are less effective in removing the water-rigor contraction than those which contain small amounts. This fact is readily explained by the considerations that alkalies as well as acids tend to cause muscle to swell, that $NaHCO_3$ solutions have a slightly alkaline reaction, and that the greatest effect in removing the swelling would therefore be obtained with a solution which contained enough $NaHCO_3$ to react with the comparatively small amount of lactic acid formed by the muscle, but not enough to produce any appreciable alkaline reaction.

A muscle which has been thrown into water rigor and then brought back to its original length by 0.7 per cent NaCl solution or by Ringer's solution may be made to shorten again by immersion in a swelling reagent. The amount of this second shortening is variable, and depends, among other things, on the time which has elapsed since the death of the muscle, and on the temperature of the fluids in which it has been kept. It has been pointed out that the ability of a body to shorten under the influence of distention depends on its possessing a peculiar structure. It is *a priori* not improbable that dead muscle should gradually lose this structure, and the fact that it gradually loses the power of shortening under the influence of distention may be taken as confirmation of this view. Fig. 9 gives an example of the shortening produced by weak lactic acid. I have experimented also with distilled water, slightly hypertonic cane-sugar solutions, HCl, NaOH, and KOH, all of which have been shown to produce swelling in dead muscle (see Figs. 3 and 6, pp. 197, 199, and Fischer, *Loc. cit.*, on p. 196). All of these reagents produce more or less shortening; that produced by the sugar solutions is slowest; by distilled water, next; while the acids and alkalies produce a comparatively rapid shortening. Unless these are very dilute, however, the shortening lasts only a few seconds, and is then succeeded by lengthening. This lengthening may go on until the muscle is much longer than its normal extended length; at the same time it loses most of its tensile strength and assumes a jelly-like consistency and a peculiar transparent appearance.

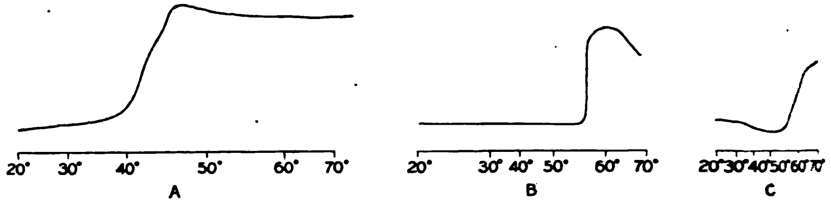

FIGURE 11. — The effects of heat on the length of fresh sartorius (A), a frog's tendon (B), and a sartorius (C) which had been treated for eighteen hours with distilled water at 20° and then for two hours with 0.7 per cent NaCl solution at 20°. The heat shortening of the dead muscle occurs at about the same temperature as that of the tendon.

It has been known for a long time that muscle fibres may be broken up into "discs" by the action of acids, and it seems highly probable that this breaking up of the muscle fibres accounts for the great lengthening and the loss of tensile strength under the influence of comparatively strong acids and alkalies. Such a process would, of course, destroy the structure which enables the sarcostyles to shorten under the influence of distention, and after its occurrence the muscle would swell in every dimension like any structureless body.

It has been shown in Part I (p. 200) that solutions of NaCl above 5 per cent cause the swelling of muscle which has been treated with distilled water. Such strong salt solutions cause muscles in water rigor to lengthen more rapidly than do 0.7 per cent NaCl solutions. But muscles which have been caused to lengthen by 5 per cent NaCl solutions cannot be caused to shorten again by distilled water, acids, and alkalies, and it is therefore highly probable that the lengthening under these circumstances is the result of rupture of the sarcostyles.

Heat has an interesting effect on muscle which has been treated for several hours with distilled water and of which the water-rigor contraction has then been partially removed by Ringer's solution. Fig. 11 C gives the effect of heating such a muscle gradually to 70° and may be compared with Fig. 11 *A*, which represents the heat curve of a fresh sartorius, and Fig. 11 *B*, which is the heat curve of a piece of frog's tendon. It will be seen that the dead muscle instead of shortening at about 40°, as does the living muscle, lengthens up to a temperature of about 55° and particularly from 35° onward. It may be shown that heating a muscle which has been treated in the manner described causes a considerable decrease in its weight. At about 55° in all three cases there appears the marked heat shortening which occurs near this temperature in almost all animal tissues.

It is a very interesting fact that a muscle in the late stages of water rigor may be made to lose water simply by stretching it. The following is the description of a typical experiment:

A frog's sartorius was immersed in distilled water for seventeen hours at 20°. At the end of this time it weighed 0.226 gm.

It was then put under a tension of 5 gm., while still immersed in the distilled water at the same temperature.

	Absolute weight. Gm.	Percentage change.
After thirty minutes it weighed	0.210	−7
After thirty minutes more	0.195	−7
After thirty minutes more	0.190	−2.6
Total loss	0.036	−16

The tension was then released, and the muscle was allowed again to lie in the water with its ends free. After thirty minutes it weighed 0.199 gm., a gain of 4.7 per cent.

This and other similar experiments leave no doubt of the fact that water may be driven out of a muscle in water rigor by tension, and that the muscle tends to go back to its original weight as soon as the tension is released. These facts again are readily explained on McDougall's hypothesis, which carries with it the corollary that the weight which can be lifted by contracting muscle depends on the internal pressure which can be brought to bear on the walls of the sarcostyles. Hence any weight stretching the muscle would increase the pressure within the sarcostyles and tend to drive fluid out of them. I was led to try this experiment and enabled to predict its result from the considerations which have been outlined.

The fact that stretching a muscle in water rigor tends to drive water out of it explains the fact that in my experiments the spontaneous reversal of the water-rigor contraction occurred earlier than the beginning of the second loss of weight (see p. 205, and Figs. 1 and 7). Those muscles in which the changes in length were followed during long periods of immersion in distilled water were always stretched by a weight of 1 gm.; while the muscles of which the changes in weight were followed were allowed to lie unstretched in the distilled water. The muscles of which the changes in length were followed, therefore, probably began to lose weight earlier than the others, and the discrepancy between the time at which the shortening is reversed and that at which the second increase in weight is reversed is only apparent.

The ideas and observations embodied in the foregoing article furnish an interesting explanation of certain facts reported by Overton, who found that muscles kept in hypotonic NaCl solutions above 0.35 per cent do not take up as much water as they should if all parts of the tissue took part in the process. He concludes that a part of the water within the muscle fibres is held by the solid constituents of the fibres in a form which he designates as "Quellungswasser," or, in other words, that some constituents of the fibres take no part in the swelling that is undergone by the muscle as a whole in such solutions.[21] I have shown that muscle immersed in hypotonic NaCl solutions above 0.3 per cent undergoes no shortening,[22] and this would indicate, according to McDougall's hypothesis, that the sarcostyles take up no water under such circumstances. It seems, therefore, quite possible that the sarcostyles are the portions of the muscle fibre which fail to take up water from the solutions in question.

General Summary.

In the first part of this article further evidence was added to that which has already been given by Overton and others to show that striated muscle is capable of two distinct kinds of swelling. The first of these is dependent on the possession by the muscle fibre of an osmotic membrane permeable to water, but not to inorganic salts and a number of other crystalloid substances; the second is most readily studied after the destruction of the osmotic membranes surrounding the fibre, and is akin to the swelling of fibrin and gelatin in distilled water, acids, and alkalies. Reasons were given for believing that the second kind of swelling is a property of the muscle sarcostyles alone.

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[21] Overton: Archiv für die gesammte Physiologie, 1902, xcii, pp. 133–142.

[22] Meigs: This journal, 1908, xxii, p. 408.

The facts which have been reported in Part II may justly be taken to show that the sarcostyles of frog's striated muscle are so constructed that any increase in their volume brings about, as a necessary mechanical result, a decrease in their length. It had already been shown that the sarcostyles tend to swell in acid solutions, and it may be shown that the condition known as rigor is always accompanied by the accumulation of lactic acid within the muscle. Fletcher and Hopkins have demonstrated this for death rigor, heat rigor, CO rigor, chloroform rigor, and the more rapid death rigor which is brought on by lack of oxygen and fatigue. Du Bois-Reymond has shown the same thing for water rigor and the rigor brought on by freezing and thawing, and I have shown it more fully for water rigor and for the rigor which is brought on by strong hypertonic solutions.[23] It may be said, therefore, that all forms of rigor are explained by the considerations above set forth; the accumulation of acid within the muscle results in a swelling of the sarcostyles at the expense of the sarcoplasmic fluid, and the shortening of these elements is the direct mechanical result of their increase in volume. The removal of rigor by salt solutions, and particularly by salt solutions which contain small amounts of $NaHCO_3$ and therefore tend to remove the acid reaction of the muscle, are also explained. And as it has been shown that the sarcostyles possess the structure attributed to them by McDougall, that the contraction of rigor is the result of the action of lactic acid on this structure, and that lactic acid is formed under the physiological conditions which bring about contraction,[24] it is difficult not to think that McDougall's main contention is correct — that the contraction of striated muscle is the result of the swelling of the sarcostyles, which, in its turn, is brought about by the formation within them of minute quantities of lactic acid.

[23] Meigs: The journal of physiology, 1909, xxxix, p. 385.

[24] Fletcher and Hopkins: The journal of physiology, 1907, xxxv, p. 247.

SOME MODIFICATIONS OF THE METHOD IN USE F DETERMINING THE QUANTITY OF MONO-AMI ACIDS YIELDED BY PROTEINS WHEN HYDROLYZ WITH ACIDS.

BY THOMAS B. OSBORNE AND D. BREESE JONES.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*[1]]

THE large deficit which even the most carefully conducted anal of proteins always show makes evident the importance o modifying the methods now in use that the yields of the several am acids may, if possible, be made more nearly quantitative. A stud the methods for determining arginine, histidine, lysine, and ammo recently made in this laboratory, showed that these give results w are in a high degree satisfactory. The chief source of the deficit, th fore, lies either in the determinations of the mono-amino-acids or in presence among the products of hydrolysis of a large proportion of unknown substances. We have consequently thought it well w while to devote considerable time to a study of some modification these methods and have compared the results obtained by appl them with those previously obtained by following, as closely as we co the directions given by Emil Fischer.

The modifications employed and the results obtained are give the present paper. A critical consideration of the methods of ana as a whole and the probable amount of each of the several amino-a actually present in the solution will be considered in another paper to follow.

APPLICATION OF PHELPS AND TILLOTSON'S METHOD OF ESTERIFYING ORGANIC ACIDS.

The first point to which we turned our attention was the proces esterifying, for it seemed possible that, by applying the method rece

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[1] The expenses of this investigation were shared by the Connecticut Agricul Experiment Station and the Carnegie Institution of Washington.

proposed by Phelps and Tillotson[2] for esterifying organic acids, not only less time and labor would be required, but also a better yield of esters might be obtained.

This method consists in continuously passing a current of the vapors of absolute alcohol through an alcoholic hydrochloric acid solution of the chlorides of the amino-acids to which is also added some zinc chloride as a catalyzer. As the solution is kept during the process at a temperature slightly above 100°, water is removed by the alcohol vapors as fast as it is formed, and theoretically a much more complete esterification should result sooner than when the water is removed by repeated evaporations with large quantities of alcohol.

In order to test this method first on the pure amino-acids we dissolved 20 gm. of leucine in 30 c.c. of alcoholic hydrochloric acid, added 80 c.c. of absolute alcohol, and 4 gm. of zinc chloride The vapors of 900 c.c. of absolute alcohol, containing 35 c.c. of alcoholic hydrochloric acid, were passed during six hours through the mixture, heated to about 105°. The esters were then set free and extracted with ether according to the method described by Fischer. After saponifying, 17.76 gm. of leucine, or 88.8 per cent of the original amount, were recovered.

Twenty grams of glutaminic acid, treated in a similar manner for four hours with the vapors of 500 c.c. of absolute alcohol, containing 18 c.c. of alcoholic hydrochloric acid, yielded 21.17 gm. of glutaminic acid hydrochloride, or 85 per cent of the theoretical. In this case the ether solution of the esters contained some zinc chloride which was separated from the glutaminic acid by hydrogen sulphide.

Twenty grams of l-proline and 3 gm. of zinc chloride were dissolved in 25 c.c. of absolute alcohol together with 15 c.c. of alcoholic hydrochloric acid, and the vapors of 600 c.c. of absolute alcohol, containing 20 c.c. of alcoholic hydrochloric acid, were passed through the solution heated to 100°–103° during five hours. From the esters thus produced there were obtained 13.9 gm. of l-proline, equal to 69.5 per cent.

As this method of esterification gave promise of success, we applied it to the products of hydrolysis of zein. Zein was selected for trial because it contains a larger proportion of mono-amino-acids than any other known protein, very little arginine and histidine and no tryptophane or carbohydrate, as shown by the absence of any reaction with the glyoxylic acid or Molisch tests. As neither tryptophane nor carbo drates can be quantitatively estimated among the products of hydrol of proteins, by using zein for our experiments all uncertainties aris from the presence of these substances are eliminated.

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[2] PHELPS and TILLOTSON: American journal of science, 1907, xxiv, p. 194.

FIRST ANALYSIS OF ZEIN.

A quantity equal to 73.5 gm. of moisture and ash-free zein was boi in an oil-bath with hydrochloric acid for sixteen hours. The wa was removed by evaporating at 40°–50° to a syrup under diminish pressure, dissolving the residue in much absolute alcohol, again evap rating to a syrup and again repeating the process. The syrup was th dissolved in 75 c.c. of absolute alcohol, 25 c.c. of alcoholic hydrochlo acid added, and also 12 gm. of zinc chloride. After heating the m ure in a flask to 105° to 110°, the vapors of 1100 c.c. of absolute alcol plus 40 c.c. of alcoholic hydrochloric acid were passed through solution during eight hours. The esters were at once shaken out in t usual way and dried over sodium sulphate for four weeks.

When distilled *in vacuo*, the following fractions were obtained:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	105°	0.6 mm.	43.54 gm.
II	100°	0.8 "	41.05 "
Total esters			84.59 gm.
Undistilled residue			12.00 "

Analysis of these esters yielded alanine and valine 4.82,[1] alanine 2. valine 1.88, leucine 10.55, proline 8.23, phenylalanine 0.55, aspartic acid 1 per cent.

SECOND ANALYSIS OF ZEIN.

The above figures, which are all in excess of those previously fou by Osborne and Clapp, show that very satisfactory results were obtain by this method and led us to apply it to a larger quantity of zein, maki two ester fractions and analyzing the esters separately. In each ca care was taken to weigh only pure products which were identified the usual methods, the details of which do not need to be repeated here.

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[1] No attempt was made to separate this mixture, as this was not important for the comparison between the results of this and other hydrolyses.

A quantity of zein equal to 367.35 gm., ash and moisture free, was hydrolyzed by heating on a boiling-water bath for thirty-two hours with 1000 c.c. of hydrochloric acid, sp. gr. 1.1, and then by boiling in an oil bath for twenty-two hours. Glutaminic acid was separated in the usual way, and 91.6 gm. of pure hydrochloride were obtained, equivalent to 73.35 gm. of free glutaminic acid. This, with the 22.78 gm. subsequently obtained from the esters, makes a total of 96.45 gm., or 26.17 per cent of the zein.

This is a very much larger proportion than was previously obtained by Osborne and Gilbert[4] or by Osborne and Clapp.[5] We therefore subjected the glutaminic acid which had been weighed to a very rigid examination for its purity and satisfied ourselves that no mistake had been made. We also made several separate new attempts to obtain a similar quantity from other portions of the same preparation of zein, but all of these yielded from 18 to 20 per cent, that is, quantities similar to those previously reported. We are convinced that glutaminic acid separates with difficulty from the mixed products of hydrolysis of zein, and that it is only by a fortunate and accidental adjustment of the conditions that we obtained so much in this case.

The first esterification. — The filtrate from the glutaminic acid hydrochloride was concentrated under diminished pressure to a syrup, the latter taken up in absolute alcohol and again concentrated to remove the greater part of the water. The residual syrup was then dissolved by adding 40 c.c. of concentrated alcoholic hydrochloric acid and 50 c.c. of absolute alcohol. After adding 35 gm. of zinc chloride the vapors of 660 c.c. of absolute alcohol, containing 20 c.c. of alcoholic hydrochloric acid, were passed during five hours through the solution which was kept at a temperature of about 107° by heating in an oil bath. The esters were then, in the usual way, set free with sodium hydroxide and potassium carbonate and shaken out with ether. After drying over sodium sulphate for one week the ether was distilled off at atmospheric pressure, and the mixed esters, which still contained a little alcohol and ether, were found to weigh 275 gm. They were then distilled at 40 mm. until most of the ether had been removed, when liquid air was used to condense all the vapors that subsequently came over. following fractions were obtained:

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[4] Osborne and Gilbert: This journal, 1906, xv, pp. 333–356.

[5] Osborne and Clapp: *Ibid.*, 1908, xx, pp. 477–493.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I (*a*)	90°	15.00 mm.	40.83 gm.
(*b*)	100°	0.55 "	51.74 "
(*c*)	105°	0.55 "	48.90 "
			141.47 gm.
II	150°	0.55 "	50.56 "
III	205°	0.60 "	17.06 "
Total			209.09 gm.
Substance condensed by liquid air			24.25 "
Residue undistilled			22.00 "
Total			255.34 gm.

The substance condensed by liquid air was saponified by bo with water and evaporated to dryness. The residue, which wei 2.01 gm., was extracted with absolute alcohol, and the extract a to the alcoholic solution later obtained from fraction I.

The residue insoluble in alcohol was added to the correspon residue of the amino-acids from fraction I.

Fraction I was saponified with water in the usual way, the sol evaporated to dryness, and the residue extracted with boiling abs alcohol. The alcoholic solution was then evaporated to dryness residue extracted with absolute alcohol, the solution worked up proline in the usual way, and the proline weighed as copper salt. the dl-proline 2.63 gm. were obtained, and of the l-proline 24.29 equal to 26.92 gm., or 7.33 per cent of the zein.

The amino-acids insoluble in alcohol weighed 71.39 gm., and subjected to fractional crystallization yielded 45.64 gm. leucine, gm. alanine, and 10.12 gm. of a mixture of valine and alanine, w contained 42.17 per cent of carbon. The losses involved in these se tions were equal to 3.04 gm.

Fraction II was shaken out with ether in the usual way, and 16.07 of phenylalanine isolated as the hydrochloride. The aqueous sol was saponified with baryta, the barium removed with an equiv quantity of sulphuric acid, and the concentrated solution satu with hydrochloric acid. There separated 10.33 gm. of glutaminic hydrochloride, which melted at 197°. The filtrate from this, when freed from chloride, yielded 2.06 gm. of aspartic acid in characteristic crystals, which reddened at 300° but did not decompose. The filtrate from the aspartic acid when boiled with copper oxide yielded 1.19 gm. more of aspartic acid as the copper salt.

After removing the copper from the filtrate from the copper aspartate, and concentrating to a small volume, 1.88 gm. of phenylalanine separated in characteristic crystals which decomposed at 270° and gave a strong odor of benzaldehyde when boiled with sulphuric acid and potassium bichromate. The filtrate from the phenylalanine on further concentration yielded 0.71 gm. of glutaminic acid in characteristic crystals which melted at 202°. The mother liquor was a syrup from which no serine could be separated. After long drying over sulphuric acid it weighed 2 gm. There were thus isolated, from fraction II, 17.95 gm. of phenylalanine, 8.99 gm. of glutaminic acid, and 3.25 gm. of aspartic acid.

Fraction III, when shaken out with ether, yielded 1.6 gm. of phenylalanine as hydrochloride. The aqueous layer, when saponified with baryta and worked up in the usual way, yielded 10 gm. of glutaminic acid hydrochloride which decomposed at 198°, and 0.88 gm. of tyrosine, which separated in characteristic crystals and had the correct composition:

Carbon and hydrogen, 0.1419 gm. substance, gave 0.3104 gm. CO_2 and 0.0709 gm. H_2O.

Calculated for $C_7H_{11}NO_3$ = C 59.63; H 6.12 per cent.
Found . . . . . . = C 59.66; H 5.59 per cent.

The presence of tyrosine has since been recognized in all the esters obtained by Phelps and Tillotson's method. The mother liquor from the tyrosine could not be made to yield any further crystalline separation. After long drying over sulphuric acid it yielded a dense syrup which weighed 1.5 gm.

The distillation residue. — This was examined in the usual way, but no glutaminic acid or other substance could be isolated from it.

The second esterification. — The products of a second esterification were examined separately, in order to determine the relative proportion of the several amino-acids which were not esterified the first time. After removing the inorganic salts and water the alcoholic solution of the hydrochlorides of the amino-acids was evaporated to a small volu 10 c.c. of alcoholic hydrochloric acid and 10 gm. of zinc chloride ad and the esterification effected by passing the vapors of 1300 c.c absolute alcohol, containing 40 c.c. of alcoholic hydrochloric a through the solution during six hours. The esters were then sha out in the usual way and dried over sodium sulphate for three we The ether was then removed at atmospheric pressure and 117 gn mixed esters obtained.

The esters were then distilled under diminished pressure with following results:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	105°	0.7 mm.	49.33 gm.
II	195°	0.7 "	27.65 "
	Condensed by liquid air		26.00 "
	Distillation residue		12.00 "
	Total recovered		114.98 gm.

Fraction I, when saponified in the usual way, yielded 29.46 gr amino-acids insoluble in alcohol and an alcoholic extract from w 6.28 gm. of proline were isolated as the copper salt.

By fractional crystallization of the acids insoluble in alcohol, gm. leucine, 4.50 gm. alanine, and 6.81 gm. of a mixture of valine alanine were obtained. The mixture of valine and alanine conta carbon 48.04 and hydrogen 8.59 per cent, and yielded no leucine lead salt when treated according to the method of Levene and Slyke.[6]

Fraction II was extracted with ether in the usual way, and 3.40 of phenylalanine were obtained as the hydrochloride from the solution.

The aqueous layer, by the usual treatment, yielded 7.80 gm. of g minic acid hydrochloride, 3.10 gm. of aspartic acid as the copper and 0.64 gm. of tyrosine. The final mother liquor yielded no s and when dried *in vacuo* for a long time over sulphuric acid wei 7.08 gm.

The distillation residue. — This was examined carefully for g minic acid in the usual way, but none could be isolated from it.

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[6] LEVENE and VAN SLYKE: Journal of biological chemistry, 1909, vi 391–417.

Examination of the substance remaining after the second esterification. — After removing the salts as completely as possible by means of alcoholic hydrochloric acid and the excess of hydrochloric acid by repeated evaporation under diminished pressure, the solution was diluted to 6 litres, 5 per cent of sulphuric acid added, and the arginine and histidine precipitated by adding 400 gm. of phosphotungstic acid. After filtering out the precipitate the solution was freed from the excess of phosphotungstic and sulphuric acids with baryta, from baryta with a slight excess of sulphuric acid, and then concentrated under diminished pressure to a small volume. The small amount of hydrochloric acid still in the solution was removed with silver sulphate and the sulphuric acid with an equivalent quantity of baryta. The solution was then concentrated at a low pressure to dryness. The crystalline residue weighed 18 gm., equal to 4.9 per cent of the original zein.

Assuming that 75 per cent of the products of hydrolysis were esterified each time and that the weight of the amino-acids was equal to that of the original zein, which is only approximately true, we calculate that, including the basic amino-acids, there should be about 23 gm. of unesterified substance in this solution. Such a calculation is only roughly approximate, but serves to show that the quantity of acids obtained from this solution is not very different from that which should be expected.

By systematic fractional crystallization of this residue 0.20 gm. of tyrosine, 1.28 gm. of leucine, or 0.35 per cent, and 3.76 gm. of serine, or 1.02 per cent, of the zein were isolated. The remainder of the substance consisted of a mixture of amino-acids from which no oxyproline or other definite substance could be obtained and behaved throughout like the mixture of amino-acids which usually results from protein hydrolysis. As care was taken to avoid as far as possible all losses in recovering the unesterified substances which remained after the second treatment according to Phelps and Tillotson's method, it is evident that relatively little of the products of hydrolysis escaped conversion into esters and that such losses as did occur are not to be ascribed to incomplete esterification.

The following table gives the results of this analysis stated in per cent of the zein, the proportion of each substance isolated from the products of each esterification being given separately in order to show the proportion esterified each time. In order to eliminate the unavoidable loss incurred in separating valine from alanine, the sum of these is given and also the total quantity of each actually isolated.

TABLE I.

Substance.	Esters I.	Esters II.	Total.
Alanine and valine . . .	5.94	3.08	9.02
Alanine	...	...	6.08
Valine	...	...	1.05
Leucine	12.43	4.50	16.93
Proline	7.33	1.71	9.04
Phenylalanine	5.32	0.92	6.24
Aspartic acid	0.89	0.84	1.73

These results show how important it is to make at least two esterifications when quantitative results are desired.

It is difficult to compare the degree of esterification by this new method with that obtained by Osborne and Clapp in their analysis of zein according to Fischer's method, for no definite basis for a fair comparison exists. The weight of undistilled esters cannot be strictly compared, because these always retain more or less ether and alcohol after the greater part of the ether is removed at atmospheric pressure, and the amount of glutaminic acid ester also differs with the amount of glutaminic acid hydrochloride removed before esterifying. If, however, we make an approximate comparison, we obtain the following results: Deducting from the sum of the distilled esters and the undistilled residue the weight of the substance condensed by liquid air, we have a total of 320.07 gm. of esters by Phelps and Tillotson's method, or 87 per cent of the zein hydrolyzed. Since, in the analysis by Fischer's method, the substance condensed by liquid air was not weighed, the total undistilled esters plus the undistilled residue may be compared with the above figures. We thus find that the esters in this case were equal to 79.8 per cent of the zein. The actual difference, however, is much greater, for in our present analysis we removed, before esterifying, 20 per cent of glutaminic acid, whereas Osborne and Clapp removed only 10.9 per cent. The amount of glutaminic acid ester must, therefore, have been very much more in the esters obtained according to Fischer's method, and the advantage of the new method correspondingly greater.

If the comparison is made between the *distilled* esters, the difference appears even more to the advantage of the new method, for the yield of such esters was equal to 77.8 per cent of the zein by Phelps and Tillotson's method and but 62.6 per cent by Fischer's method, the difference being equal to nearly 25 per cent of the latter quantity. By this method of comparison the uncertainties caused by differences in the proportion of glutaminic acid first removed are to a large extent eliminated, but, on the other hand, differences are introduced due to a more successful distillation in one case than in the other. Thus the distillation residue in this present analysis was equal to 9.3 per cent of the zein, whereas in that made by Osborne and Clapp it was equal to 17 per cent. It does not seem to us probable that the yield of esters by Phelps and Tillotson's method should necessarily be greater than that by Fischer's, but that such advantages as it appears to show, as in this case, are to be attributed to the greater ease with which it can be con-

TABLE II.

Substance.	Osborne and Jones.	Osborne and Clapp.[1]	Substance.	Osborne and Jones.	Osborne. and Clapp.[1]
Valine and alanine	1.89	...	Serine	1.02	0.57
Alanine	6.08	2.23	Tyrosine	3.55	3.55
Valine	1.05	0.29	Arginine	1.35 [3]	1.16
Leucine	18.30 [2]	18.60	Lysine	0.00	0.00
Proline	9.04	6.53	Histidine	0.82 [3]	0.43
Phenylalanine . . .	6.22	4.87	Tryptophane . .	0.00	0.00
Aspartic acid . . .	1.71	1.41	Ammonia	3.64 [3]	3.61
Glutaminic acid . .	26.17	18.28	Total	80.74	61.53

[1] *Cf.* OSBORNE and CLAPP: This journal, 1908, xx, p. 484.

[2] Includes 1.02 per cent isolated from the crude glutaminic acid hydrochloride.

[3] OSBORNE, LEAVENWORTH and BRAUTLECHT: This journal, 1908, xxiii, pp. 180–200.

ducted. There is no apparent reason why the yield of esters shoul not be practically the same by either method.

The convenience of this new method of esterifying is so great that w have employed it in all our subsequent work.

In Table II we give the results of this new analysis of zein, an also include determinations of arginine and histidine made in thi laboratory since the publication of the earlier analysis by Osborne an Clapp.

A comparison of these results shows that larger quantities of eac of the amino-acids, which were separated from the esters made by th new method, were obtained except of leucine, and that a large part the deficiency shown in the earlier analysis is thus accounted for.

Although the actual quantities of nearly all of the amino-acids isolate in this new analysis of zein are considerably greater than those formerl obtained, the general picture presented by these two analyses is muc the same, and it is probable that those analyses of other proteins whic foot up over 60 per cent, while representing the quantities of amin acids as much less than they actually are, present a general idea of th relative differences between the proteins which is of much value.

THIRD ANALYSIS OF ZEIN.

In distilling the esters of the products of protein hydrolysis more less decomposition becomes evident as the temperature of the distillin solution rises. In consequence a not inconsiderable quantity of alcoho is condensed by the liquid air and also small amounts of sulphur-containing and other products. We have accordingly thought it wort while to find out whether or not the losses which must inevitably aris from this decomposition cannot be materially reduced by terminatin the distillation after the leucine ester has come over, which usually i the case when the oil bath reaches 110° and the pressure is below 1 mm. and then treating the residue of undistilled esters in the same way a the esters which distil above this temperature are commonly treated.

The first esterification. — Another analysis of zein was therefore mad with this object in view, and also to learn how close an agreement ca be expected between several analyses of the same protein, for evidenc along these lines is as yet scanty.

For this analysis a quantity of air-dry zein, equal to 383.5 gm. of th moisture, fat, and ash-free substance, was heated with 1000 c.c. of hydrochloric acid, sp. gr. 1.1, first at 100° for twenty hours and then boiled in an oil bath for thirty-seven and one-half hours. After removing the excess of hydrochloric acid by twice evaporating under diminished pressure with much alcohol, the esterification was effected by dissolving the syrup in alcohol to which 10 c.c. of alcoholic hydrochloric acid and 50 gm. of zinc chloride were added. The vapors of 3000 c.c. of absolute alcohol, containing 90 c.c. of alcoholic hydrochloric acid, were then passed continuously for ten hours through the solution heated to 108°. The esters were set free with sodium hydroxide and potassium carbonate in the usual way and shaken out with ether. After drying for several days over sodium sulphate the ether was distilled off at atmospheric pressure. The yield of crude esters was 378 gm., which is much more than in the preceding analysis, because in this case the glutaminic acid had not been removed.

The esters were then distilled at a pressure of 0.2 mm. up to 110° temperature of the bath. The total weight of the distillate was 124.63 gm., which is less by 16.84 gm. than that obtained up to 105° in the preceding analysis.

During the distillation liquid air was used from the outset in order to condense everything that came over. The liquid so condensed weighed 10 gm., but when saponified yielded only 1.05 gm. of crystalline substance, which was added to the products obtained by similarly saponifying the distilled esters. It thus appears that the greater part of substance condensed by the liquid air was alcohol and ether which had not been completely removed from the esters.

In order to avoid any losses that might be occasioned by subjecting the remaining esters to a higher heat, the distillation was stopped at a temperature of 110°, and the undistilled residue, which weighed 234 gm., was treated directly in the same way as Fischer directs for the treatment of the distillates obtained above 110°.

Treatment of the distilled esters. — After saponifying in the usual way, the residue which was left by evaporating the solution of the amino-acids to dryness and extracting with alcohol, until proline was removed, weighed 74.13 gm. By systematic crystallization this yielded 56.38 gm. of leucine, 8.14 gm. of alanine, and a mixture of 6.73 gm. from which, by means of the lead salt method, 3.31 gm. of leucine were isolated. The remainder of this substance was united with a similar mixture of valine and alanine obtained from the second esterification, and bot worked up together.

The alcoholic extracts from the amino-acids, which had been free from all that was insoluble in absolute alcohol, yielded 6.89 gm. racemic proline and 8.07 gm. of l-proline. These were weighed as th copper salts.

Treatment of the undistilled esters. — These were mixed directly wit 2 volumes of water, and the mixture shaken with an equal volume ether. The ether solution, which separated readily, was nearly blac The aqueous layer was shaken again with 150 c.c. of ether, and th ether solution was added to the first. The color was thus almost com pletely removed from the aqueous solution.

The ether solution was then allowed to evaporate at the room tem perature, concentrated hydrochloric acid added to the residue, and th ester saponified by heating on the water bath. A considerable quantit of oil which separated was removed by ether, and a dark-colored aqueou solution obtained which was decolorized by treating with bone coa Phenylalanine hydrochloride separated, on evaporating, in charac teristic crystals, which when recrystallized were pure and weighe [illegible] gm.

The aqueous layer from which the ether solution of the phenylalanin had separated was saponified with baryta in the same way as if th esters had been distilled. By proceeding as Fischer directs, there wer obtained from it [illegible] gm. of glutaminic acid, as the hydrochlorid [illegible] gm. of tyrosine, and [illegible] gm. of aspartic acid as the copper sal After removing the copper from the filtrate from the copper asparta and concentrating the solution to a small volume, [illegible] gm. of glutamini acid separated on long standing. No serine could be isolated from th filtrate from the glutaminic acid.

This method of isolating the amino-acids commonly obtained from the ester fractions distilled at pressures below 1 mm. ha many advantages, especially in saving time and labor. The separation are as easily effected as from the distilled esters and the products ob tained are as easily purified. The only difficulty encountered was i bringing the copper aspartate to separate, but after long standing th [illegible] the same amount as was fo [illegible] method. We have adopted this trea in our subsequent hydrolyses and have encountered no difficulty i its use. It is probable that serine cannot be so easily obtained from the greater quantity of substance thus treated as from the smaller amount of distilled esters, but as we have obtained very little serine and usually none whatever from these esters, this is not a serious disadvantage when working with vegetable proteins. Furthermore, a quantitative determination of serine appears to be impossible by the ester method, for this amino-acid is esterified with such difficulty that we have usually found it in apparently greater quantity in the mixture of unesterified products than in the esters.

The second esterification. — After removing the inorganic salts with alcoholic hydrochloric acid and the water by repeated evaporations with alcohol at a low pressure, the residual syrup was esterified by dissolving in alcohol, adding 10 c.c. of alcoholic hydrochloric acid and 14 gm. of zinc chloride and passing the vapors of 1750 c.c. of absolute alcohol and 60 c.c. of alcoholic hydrochloric acid through the solution during nine and one-half hours. The esters were set free and extracted with ether, as after the first esterification, and, after drying for some time over sodium sulphate, were distilled at a pressure of 0.38 mm. up to 105° of the oil bath.

The crude esters weighed 71 gm., the distilled esters 26 gm., the substance condensed by liquid air 8 gm., and the undistilled residue 36 gm.

Treatment of the distilled esters. — These were saponified in the usual way, the solution evaporated to dryness, and the residue extracted with boiling absolute alcohol. The amino-acids insoluble in alcohol weighed 13.33 gm. By systematic recrystallization these yielded 8.44 gm. of leucine, 0.85 gm. valine, and a mixture of valine and alanine which together with the similar mixture from the first esterification gave 2.41 gm. of valine and 4.98 gm. of alanine.

The alcoholic extract of the amino-acids, when freed from everything insoluble in absolute alcohol, yielded 11.71 gm. of proline as the copper salt.

Treatment of the undistilled residue. — This was worked up in the same way as the corresponding residue from the first distillation. The ether extract yielded 0.93 gm. of phenylalanine; the aqueous solution, 10.78 gm. of glutaminic acid, as the hydrochloride, no copper aspartate, and 1.0 gm. of tyrosine. Nothing further could be obtained from it.

The unesterified substances remaining after the second esterification were separated from inorganic salts by treating with alcoholic hydrochloric acid and evaporating several times. The solution was then diluted to 3 litres and freed from bases with 200 gm. of phosphotungstic acid in 2.5 per cent sulphuric acid solution. After quantitatively removing the sulphuric and phosphotungstic acids with baryta and the residual chlorine with silver sulphate, the solution was concentrated under diminished pressure and the semicrystalline residue dried in the flask and found to weigh 20 gm., or 5.2 per cent of the zein, as against 4.0 per cent in the preceding analysis. No attempt was made to separate definite products from this residue.

In the following table we give the results of this analysis and for comparison those of the preceding, the proportion of the amino-acids recovered in each analysis from the first and second esterifications being stated separately in per cent of the zein.

TABLE III.

Substance.	Second Analysis.			Third Analysis.		
	Esters I.	Esters II.	Total.	Esters I.	Esters II.	Total.
Alanine and valine	5.94	3.08	8.98	3.01	0.95	3.96
Alanine	...	...	6.08	. .	...	3.42
Valine	...	...	1.05	...	...	0.85
Leucine	12.43	4.50	17.95[1]	15.56	2.20	17.76
Proline	7.33	1.71	9.01	4.06	3.05	7.11
Phenylalanine . . .	5.32	0.92	6.23	6.13	0.24	6.37
Aspartic acid . . .	0.89	0.84	1.73	1.04	0.00	1.04
Glutaminic acid . .	...	...	26.17[2]	16.83	2.81	19.64[3]

[1] Includes 1.02 per cent which separated with the glutaminic acid.

[2] Separated chiefly as hydrochloride. [3] Separated from the esters.

This table shows that under the conditions prevailing in each analysis a very considerable quantity of each of the amino-acids which escaped esterification the first time was recovered by a repetition of this process. The ratio of amount obtained from the second esters to that from the first is different for the several acids in the two analyses, indicating a difference in the ease with which they are esterified under slightly different conditions or else a difference in the completeness with which the esters are extracted by the ether after the first esterification.

In the following table the results of these several analyses of zein are brought together so that they may be more easily compared:

TABLE IV.

Analyses	1	2	3	Osborne and Clapp.
Valine and alanine	4.82	1.89	...	...
Alanine	2.94	6.08	3.42	2.23
Valine	1.88	1.05	0.85	0.29
Leucine	19.55	18.30	17.76	18.60
Proline	8.23	9.04	7.11	6.53
Phenylalanine	6.55	6.22	6.37	4.87
Aspartic acid	1.34	1.71	1.04	1.41
Glutaminic acid	...	26.17[1]	19.64[2]	18.28
Serine	...	1.02	...	0.57
Tyrosine	...	3.19	...	3.55
Arginine	...	1.55	...	1.16
Lysine	...	0.00	...	0.00
Histidine	...	0.82	...	0.43
Tryptophane	...	0.00	...	0.00
Ammonia	...	3.64	...	3.61
		80.38		61.53

[1] Separated chiefly as hydrochloride.
[2] Separated entirely from the esters. This is 75 per cent of the amount separated directly, and is approximately the same proportion as is usually thus obtained.

A striking difference is shown by the figures here given for alanine and valine, which is almost certainly due, to a very large extent, to incomplete recovery of these amino-acids from their esters. We are no investigating the losses involved in this separation and expect soon t obtain more definite data in respect to this question.

The figures for leucine agree as closely as we should expect con sidering the conditions of its isolation. It is to be noted that this is th only amino-acid which was obtained in as large proportion by the Fische method of esterifying as by that of Phelps and Tillotson.

The proportion of proline shown by the three new analyses differ considerably, but considering the uncertainties which attach to this de termination the agreement may be considered to be fairly satisfactory.

The new determinations of phenylalanine are in remarkable agree ment, and show that this substance can be separated from the ester undistilled at 110° quite as completely as from the distilled esters.

Practically the same proportion of aspartic acid was obtained in eac of the four analyses, although a slightly smaller amount was obtaine from the undistilled esters, due to the slowness with which the coppe aspartate separates from the more complicated mixture.

The amount of glutaminic acid obtained from the esters in analys 3 is 75 per cent of that which was mostly separated directly as the hydr chloride in analysis 2. This is in full agreement with past experienc for the yield by the ester method is commonly about 75–80 per cent of that which is obtained as hydrochloride. That more glutaminic ac was thus obtained wholly from the esters than directly as hydrochlori by Osborne and Clapp shows that in the latter case the separation w incomplete, and also indicates that the much larger proportion foun in analysis 3 is approximately correct.

THE EXCHANGE OF AIR IN THE EUSTACHIAN OR GUTTURAL POUCHES OF THE HORSE.

By PIERRE A. FISH.

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THE Eustachian or guttural pouches are membranous sacs communicating with the pharynx on either side. The mucous membrane of the sacs is continuous with that of the Eustachian tubes, and there is a direct communication between the air of the guttural pouch and the middle ear. According to Strangeway, the guttural pouches are peculiar to the soliped and the Hyrax.

The relation of the pouches to the pharynx would apparently incline one to the view that inasmuch as the lungs are filled with air during inspiration, so might these pharyngeal appendages fill up and, like the lungs, empty themselves during expiration. Experimental evidence, however, shows that air enters the pouches during expiration.

The only previous experimental work along this line that I have been able to find recorded is that of Perosino[1] of Turin (1853), who inserted a cannula, connected with an alcohol manometer, into the guttural pouch of the horse and observed that the alcohol rose in the distal arm of the manometer with each expiration. From his experiments and general observation Perosino concluded that the pouches were filled with warm air at expiration, and during inspiration a portion of the warm air in the pouches streamed into the pharynx and mixed with the incoming cold air and modified the temperature and force of the current.

In the following work a number of horses, under chloroform anesthesia, were experimented upon in various ways. In some instances a pneumograph, connected with a tambour, was applied to the region of the flank where the respiratory movements were most pronounced. This method, after a few trials, was not regarded so satisfactory as when a cannula was inserted directly into the trachea. The latter method was selected for the most of the experiments. A tambour connected with a cannula was used for the tracing from the pouch. In most cases the cannula was inserted into the pouch externally near the inferior border of the parotid gland (Viborg's operation). In a few cases the cannula was passed into the pouch through the natural opening from the pharynx. A manometer was also used in connection with the pouch in place of the tambour, but the latter was found more satisfactory.

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[1] Von Prince: Abstract in Repertorium der Thierheilkunde von Prof. E. Hering, Sechszehnter Jahrgang, 1855, pp. 53-54.

The recording lever of the respiratory tambour and that of the pouch tambour were placed in as exactly the same vertical plane as possible upon the smoked drum, and the tracings were then taken synchronously, the one above the other.

When the cannula was inserted into the guttural pouch through its walls and the tracing obtained from the pouch compared with that taken from the trachea, it will be observed that the two tracings are very similar (Fig. 1). At the beginning of expiration the guttural pouch fills up rapidly with the expired air, a considerable portion of which immediately passes out again during the expiratory pause. Inspiration probably completes the transfer of the air to the pharynx, but, as a rule, the larger volume has escaped during the pause. This result is at variance with the conclusions of Perosino, who considered that the air was expelled during inspiration. This would be a natural assumption; but the fact that it was possible to introduce the finger into the Eustachian orifice in some of the experiments and to feel quite a forcible contraction of the parts upon it, indicated that the orifice closed during inspiration and relaxed during expiration. There is apparently a reversed analogy to the glottis in its opening and closing during inspiration and expiration. It was not determined whether or not the closure of the Eustachian orifice was air-tight, but the pressure upon the finger was sufficient to indicate that it might be.

If the cannula of the pouch tambour was introduced into the pouch through the Eustachian orifice, it was observed that the levers of the two tambours did not rise and fall together as in the former experiment, but that the pouch lever rose while the lever connected with the trachea was falling; when the tracheal lever rose, the pouch lever fell.

Indeed the levers, in meeting and receding, had much the same relation as the blades of a pair of scissors when they are opened and closed (Fig. 2).

A model to represent the pharynx and guttural pouches was prepared by using a glass tube for the pharynx. On each side of the tube was an opening around which an elastic sac was secured. At one end of the glass tube a rubber bulb was attached to represent the lung as it expanded and contracted. With this apparatus it was easy to demonstrate that the lateral sacs filled up with each compression of the bulb corresponding to the expiratory phase of respiration.

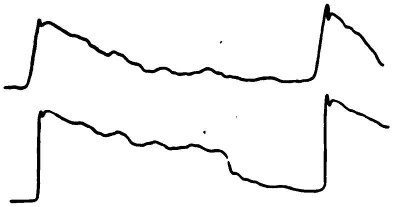

FIGURE 1. — About one half the original size. The lower tracing was taken from the trachea of the horse. The upper tracing was taken through the wall of the guttural pouch. The levers of the tambours rose and fell together, and the similarity of the tracings is marked.

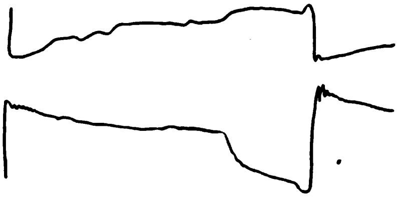

FIGURE 2. — About one half the original size. The upper tracing was taken from a cannula inserted into the orifice of the guttural pouch. The lower tracing was taken from a cannula inserted into the trachea. The levers were in opposing phases; as the respiratory lever fell, the pouch lever rose.

Various ideas as to the functions of the guttural pouches have been advanced. The view has been maintained that they are accessory organs to the Eustachian tubes and belong to the auditory mechanism; that they serve to check the rapid current of air utilized for the purpose of renewing that in the cavity of the middle ear and to balance the column of air which impinges against the tympanic membrane, — practically to serve as reservoirs in maintaining the constant pressure of air on the tympanic membrane. Other animals, however, with the sense of hearing as highly developed as the horse do not possess these pouches.

Another function ascribed to them is that they may serve as resonating chambers and assist in perfecting the sounds of neighing somewhat similarly to that of the ventricles of the larynx. Again, other animals with phonation as highly developed as the horse do not possess these sacs.

Prangé has advanced the view that the guttural pouches are s
air containers, serving as elastic cushions, through which the m
less violent movements of the head upon the neck are taken u
neutralized.

Regulating or modifying respiration is another function ascri
the pouches, since they alternately fill and empty themselves. I
way they may serve to deviate and diminish the force of the large v
of inspired air when the horse is running rapidly, and alleviate a
cessive force exerted by the air against the bronchi and the air s
the lungs. Yet other animals not possessing these pouches can
as rapidly as the horse.

Still another view is that the temperature of the inspired air is
fied or increased in warmth by the admixture with it of the ai
the pouches. Even if it be admitted that the air streams out fro
pouch during inspiration and mingles with the inspired air, the v
of the former is so slight in comparison with that of the latter, th
function in increasing the temperature of the inspired air could
but slight importance.

Under certain pathologic conditions the pouches may becom
panitic, or filled with pus, and in this condition fill up the phar
cavity sufficiently to produce marked dyspnœa.

Although the hypotheses relating to the function of the pouch
not beyond criticism, the following facts seem worthy of em
It is obviously an advantage to the animal that the mucosa of th
ties of the pouch, Eustachian tube, and middle ear should come i
tact with air already warmed to the temperature of the body
than with inspired air which at best is only incompletely warmed.
ther, it is an advantage that the pouches should be more or less co
during inspiration so that the passage of air through the pharynx
lungs may be facilitated.

THE REACTIONS OF THE VASOMOTOR CENTRE TO SCIATIC STIMULATION AND TO CURARE.[1]

By TORALD SOLLMANN and J. D. PILCHER.

[From the Pharmacological Laboratory of the Medical Department of Western Reserve University, Cleveland, Ohio.]

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I. Introduction.

THE reactions of the vasomotor centre are commonly studied by the changes of the blood pressure, organ-volume, or vein-flow. These methods render good service if the condition to be investigated can be confined strictly to the vasomotor centre. Very often this is not the case, and then these methods fail to give trustworthy information. A simultaneous cardiac effect may so actively overshadow a vasomotor action that none of the customary methods can indicate what changes may be taking place in the vessels. This often happens in certain types of asphyxia. If the cardiac element can be excluded and the change is entirely vascular, it is easily possible to distinguish whether the vasomotor effect is purely central, or purely peripheral. But if a drug, for instance, either paralyzes or strongly stimulates the vessels peripherally, this would entirely obscure whatever action it might have on the centre. This question arises with epinephrin and with the nitrites.

Again, it is often a very difficult matter to decide whether an observed vascular change in an organ is the direct result of alterations

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[1] A preliminary communication on this subject was read at the Boston meeting of the Society for Pharmacology and Experimental Therapeutics.

in its own vascular tone, or whether it is simply the mechanical resu
of the displacement of blood by the alterations in the vascular to
of other organs. With hemorrhage and transfusion, particularly, t
question arises constantly how far the alterations in the qua
tity of blood may be responsible, mechanically, for the observ
phenomena.

For many purposes, therefore, it is very desirable, and even ind
pensable, to obviate these complicating factors.

This may be done in a very simple manner, namely, by the ar
ficial perfusion of an organ; the arterial connections being complete
severed, and the nervous connections being left intact. The separ
tion of the organ from the circulation of the animal removes it entire
from the hydrostatic influence of cardiac and mechanical change
nor can drugs, etc., which may be administered, reach the organ a
therefore its vessels or vasomotor endings. The circulation of t
organ under these conditions will still be controlled by the vasomot
centre and by the vasomotor ganglion; and by any influence whi
may act upon these; but otherwise it will be completely independe
of the animal. The ganglia need only be considered in some spec
cases. Where they do not need to be considered, the circulation
an organ so arranged reflects the reactions of the vasomotor cen
in an absolutely pure form.

This permits the study of a large number of interesting proble
on which we have been engaged for several years. The method, wh
it is extremely simple in theory,[2] is rather tedious and time-consu
ing in practice. Many experiments fail entirely on account of inju
to the nerves or to the vulnerable vasomotor centre; and even in su
cessful experiments the number of observations is more or less li
ited. For this reason our program is not yet completed, and we c
at this time discuss only a few of the problems which we have attack

The method can be applied to practically any organ. So far
have confined ourselves mainly to the spleen, checking the results

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[2] Since we have been using the method, it has been employed independen
by several other investigators for the study of restricted problems (PORTER a
PRATT, Proceedings of the Physiological Society, January, 1908; EYSTER a
WILDE, Journal of pharmacology, 1910, i, p. 308). The principle is so obvi
that we would expect that it has been used incidentally in the past, although
have not encountered it in the literature.

some extent on the kidney. The choice of the spleen was mainly for technical reasons; but it forms a part of the important splanchnic system, and it appears to be fairly representative of the splanchnic vasomotor reactions.

We have not attempted to distinguish the rôle of the constrictors and dilators. This appeared to us as an unnecessary complication, since it is commonly accepted that the constrictors dominate the vasomotor reactions, especially in the splanchnic area.

DESCRIPTION OF THE METHOD.

The animals were anæsthetized by the usual methods (for dogs, morphin, 0.01 per kg. supplemented by ether; for cats, the morphin, atropin, urethane mixture [3]). Dogs were used almost exclusively.

Tracheotomy was always performed. Curare was often used to immobilize the animal. Respiration was then practiced by the continuous insufflation of oxygen by the modified Hirsch method.[4] The vagi were usually divided early in the experiment.

The blood pressure was recorded from the carotid artery; the mean pressure by a damped mercury manometer, the excursions by a Harvard Hüerthle manometer.

The damped mercury manometer presents the very great advantage that the mean pressure can be read directly from the tracing, without the need of any calculations. Both manometers are connected with the same arte as follows: The arterial cannula connects with a T piece. One limb go to a reservoir of half-saturated magnesium sulphate, suspended four fe above the table, which serves to establish an initial pressure in the syste and for the flushing out of clots. (This, of course, is clamped before t artery is opened.) The other limb connects with a second T piece. O limb of the latter goes to the membrane manometer, the other to the mercu manometer. Between the T and the mercury manometer is a screw clam which is adjusted so that the movement of the mercury column is only ju perceptible, with the ordinary beats. All the connections are filled with t magnesium solution. The damping of the mercury manometer lessens t movements of the fluid in the system so much that magnesium effects a reduced to a minimum, and clotting is of rare occurrence.

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[3] SOLLMANN, HANZLIK, and PILCHER: Journal of pharmacology, 1910, i, p. 411.

[4] *Modified Hirsch Method.* — The principle of the continuous insufflation respiration appears to have been first introduced by HANS HIRSCH (Dissertation, Giessen, 1905; Biophysisches, Centralblatt, i, No. 1403).

We have found it advantageous to modify the details of his method. In our modification, the oxygen goes from the steel tank through a wash bottle to a flexible catheter of goose-quill size. The catheter is first pushed through the (glass) tracheal cannula so that the point lies about half an inch above the bifurcation. The return flow is thus as free as possible, and if the chest is opened, the lungs are seen to be scarcely distended. This is of particular value in taking cardiac tracings. The flow of oxygen is regulated according to the size of the animal. Usually the bubbles should be almost continuous. The color of the blood in the carotid artery or in the lungs is the best index of the efficiency of the respiration. Hirsch contended that life could not be maintained by a continuous current of air; but MELTZER and AUER (The journal of experimental medicine, 1909, xi, p. 622) have demonstrated his error. Where compressed air is installed, it could doubtless take the place of the oxygen in our modification.

The spleen is exposed through a small incision. The largest arteri branch and its accompanying vein and nerves are reserved, and the remaining vessels and tissues are tied off in two masses by stro ligatures. The reserved artery and vein are then cleaned with blu needles. It is essential, of course, that the nerve fibres be careful separated from the vessels, with the least possible injury.

The perfusion cannula is then tied into the artery and the perfusi started. When the spleen has been somewhat flushed, the outflo cannula is placed into the vein. Both cannulas point toward the splee The outflow cannula is connected with the stromuhr.

For perfusion we employ Locke's fluid, without glucose and witho oxygen — we have not found these necessary. The fluid flows from Mariotte reservoir, suspended two to four feet above the animal, a is warmed by passing through a Woulf's bottle which fits into opening of the water-heated, double-walled copper trough which use as an operating table. The Woulf's bottle has the further adva tage that it intercepts any air or sediment which may find its w into the fluid.

To record the vascular changes of the spleen, the outflow of t vein is measured, the outflowing liquid being rejected. We tried number of devices, but finally used a Ludwig stromuhr.[5] The use oil being, of course, superfluous, the emptying bulb is allowed to with air through the open tube at the top. (Instead of the regu

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[5] We are now using the highly convenient outflow bucket described by Wm. Williams, Journal of pharmacology and experimental therapeutics, 1910, i, p. 4

Ludwig bulb, we have employed one which carried three sets of bulbs, of increasing capacity, one above the other; the lowest bulb being used when the rate of flow was very slow; or all the bulbs if it was very rapid.) A slowing of the outflow corresponds to constriction, a quickening to dilation. However, as explained by Burton-Opitz,[6] constriction may temporarily quicken the flow by squeezing out the large volumes of blood which the spleen contains; and a dilation has the opposite effect. These preliminary changes are very short and can scarcely be misinterpreted. The stromuhr was connected with a signal recorder in the usual manner. The kymographion moved at the rate of 1.5 to 3 cm. per minute.

The integrity of the vasomotor connection must be controlled at intervals, if necessary, by stimulation of the sciatic, or in non-curarized animals, by connecting the tracheal cannula with a rubber tube five feet long. As is well shown in Burton-Opitz's work,[7] a few intact nerve bundles are sufficient to secure a good contraction; so that there is little chance of spoiling the experiment by injury of the nerves in the operation.

During the course of the experiments, which extended generally over one or two hours, there was usually little or no loss of excitability in the spleen itself. On the other hand, the vasomotor centre is easily injured by any serious fall of blood pressure.

The vasomotor reactions of the spleen. — These have formed the subjects of many investigations. The papers of Roy,[8] Schaefer and Moore,[9] and Burton-Opitz [10] summarize the other literature.

We found, as did all of these investigators, that stimulation of the splenic or splanchnic nerves, as also reflex stimulation through the sciatic or central vagus, causes vascular contraction. Roy denied the existence of any dilator fibres. Schaefer and Moore thought that they could demonstrate them very exceptionally, and under special conditions. But for practical purposes, the vasomotor innervation of the spleen may be considered exclusively constrictor. Roy also

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[6] Burton-Opitz and Lucas: Archiv für die gesammte Physiologie, 1908, cxxiii, p. 592.

[7] Burton-Opitz: Archiv für die gesammte Physiologie, 1909, cxxix, p. 212.

[8] C. S. Roy: Journal of physiology, 1880, iii, p. 203.

[9] Schaefer and Moore: *Ibid.*, 1896, xx, p. 1.

[10] R. Burton-Opitz: Archiv für die gesammte Physiologie, 1909, cxxix, p. 189.

believed that the vagus carried centrifugal constrictor fibres, but t
is denied by Schaefer and Moore.

The presence of the muscular tissue in the trabeculæ and caps
of the spleen deserves special consideration. Roy and Schaefer a
Moore described regular rhythmic contractions covering the period
about a minute. Burton-Opitz's stromuhr failed to reveal these va
ations. In our records, also, they are extremely rare. This ne
hardly be surprising since Schaefer and Moore found them mu
weakened in artificial perfusion with diluted defibrinated blood.
they occur at all, they are evidently too weak to affect the outflo
The last named authors showed that the rhythm is of periphe
origin; it could not therefore be affected directly in our metho
Whether or not the contractions of these muscles is also under cent
control, is not decided. It is rather improbable, but provisionally
enjoins some caution in interpreting results obtained on the sple
In our work, however, the results on the kidney furnish us with
means of control.

Roy also found that the stimulation of constrictor nerves co
tracted the spleen much more when it was distended than when
was constricted. This doubtless also influences the quantitati
change in the outflow, but apparently to a lesser degree. The qualit
tive response, of course, would not be affected.

II. THE EFFECTS OF CURARE.

Curare was employed in many of our experiments to secure imm
bility. It is therefore necessary to begin with the discussion of
vasomotor actions and of the possibility of its interference with t
vasomotor reactions to other conditions. We employed Merck's test
curare in the routine dose of 3.5 mg. per kilogram body weight (2.3 c
of a 0.5 per cent solution), injected rather quickly into the femo
vein. This is generally just sufficient to arrest respiration and mu
cular response to sciatic stimulation; in a few animals there is stil
slight, practically negligible response. The insufflation of oxyg
was started shortly before the curare was injected.

The effect on the blood pressure is typical (Figs. 1 to 3). T
curare, when quickly injected, produces a very considerable and prom

fall of the mean blood pressure, with somewhat slower but still rapid recovery. The final pressure is very slightly above the original. The vein flow from the perfused spleen is somewhat slowed, showing a moderate stimulation of the vasomotor centre. This stimulation generally sets in while the pressure is falling; but exceptionally there may be slight dilation during the fall. The constriction generally persists with slightly diminished force during the recovery of the blood pressure; or it sets in at this time if there was dilation during the fall.

FIGURE 1.

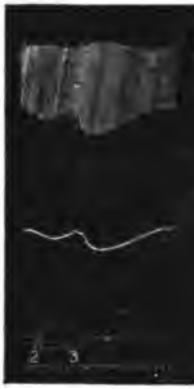

FIGURE 2.

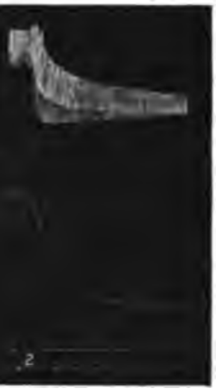

FIGURE 1. — *Curare* (Exp. C. 42-2). In all the figures, the uppermost tracing is from the Hüerthle manometer; the second, from the damped mercury manometer, shows the mean pressure. The upper base line traces the abscissa for the mercury manometer and the signals. The lowest line shows the rate of spleen-flow, *i. e.*, the revolutions of the stromuhr. The drum moves at the rate of about 2.5 cm. per minute. In this figure the oxygen was started at 2, and the curare at 3. The Hüerthle tracing is of the type A. Note the slowing of the spleen-flow.

FIGURE 2. — *Curare* (Exp. C. 33-1). Oxygen and curare at 2. Hüerthle type B. Spleen-flow slowed. The figures are one third the original size.

The fall of pressure, as read from the damped mercury manometer, averages 44 mm. Hg, or more properly, 45 per cent; the percentile fall being about the same for all blood pressures within ordinary limits (Table I). The minimum pressure is reached in one to four minutes (usually one and a half minutes). From there, the recovery requires two to fifteen minutes. The pressure after recovery averages 6 mm. above the original level. The slowing of the splenic outflow averages 17 per cent. The relative frequency and extent of the constriction during the fall and in the recovery are shown in Tables II and III.

The central vasoconstriction which is observed with the curare may be due to a slight asphyxia from the altered method of respiration, or it may be a direct expression of the strychnin-relationship of curare. In any case, it is evident that the curare fall cannot be referred to central vasomotor depression. Cardiac depression is also absent in most of the animals (as judged from the Hüerthle tracings), and the curare fall averages 35 mm. in these animals in which the

TABLE I.

INFLUENCE OF THE INITIAL BLOOD PRESSURE ON THE CURARE EFFECTS.

Level of the original blood pressure. (mm).	Number of experiments.	Average fall of pressure (mm.).				Pressure after recovery.	Rise i mm.
		From	To	=mm.	=%		
55— 70	3	64	35	29	45	68	4.0
80—105	9	97	62	35	36	97.5	0.5
110—125	10	115.5	54	61.5	53	120	4.4
130—160	6	143	81	62	43	149	6.0

TABLE II.

RELATIVE FREQUENCY OF CONSTRICTION AND DILATION (SPLENIC OUTFLOW).

During the curare fall.	During the recovery there was		
	Further constriction.	Less constriction.	Dilation.
Fifteen animals showed constriction.	1	4	0
Seven animals showed dilation . . .	4	0	0
Six animals showed no effect	3	0	1

TABLE III.

AVERAGE RATE OF SPLEEN-FLOW.

As compared with the flow before the curare, the rate of flow was:	In the animals which reacted by constriction.	In animals which reacted by dilation.	In all animals.
During the curare fall	per cent. 83	per cent. 116	per cent. 97.5
During the recovery	92	91	91

cardiac excursions were not diminished. We must therefore attribute the fall to a direct peripheral vasomotor depression, which disappears again very promptly. The return of the blood pressure, and therefore the disappearance of the vascular effect, goes hand in hand with the development of the paralysis of the skeletal muscles. One can scarcely escape the impression that the curare is taken up into the first muscles which it encounters, namely, those of the blood vessels; but that it has a greater affinity for the skeletal muscular structures and therefore is rapidly drawn into these.

FIGURE 3. FIGURE 4.

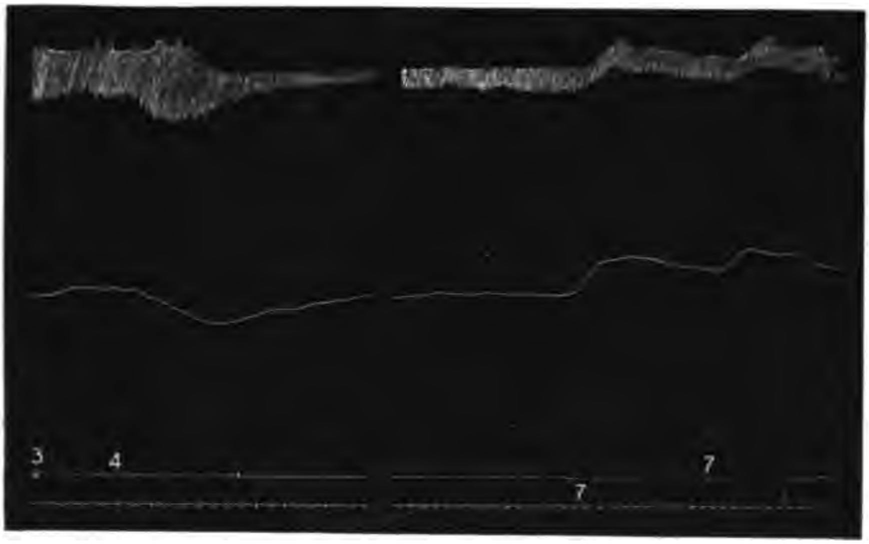

FIGURE 3.—*Curare* (Exp. C. 36–1). Oxygen at 3, curare at 4. Hüerthle type C. The spleen-flow is quickened.

FIGURE 4.—*Typical Sciatic Stimulation after Curare.* (Exp. C. 36–1). The tracing is a direct continuation of Fig. 3. The sciatic was stimulated at 7.

The previous division of the vagi does not alter the phenomena materially (Table IV). The injection of ***atropin*** (1 mg. per kg.), however, makes the curare fall considerably less marked (averaging 22 per cent instead of 46 per cent); the secondary rise is also larger. The effect on the flow is the same as in non-atropinized animals. We do not, at this time, offer any explanation for this peculiar phenomenon, but have excluded the atropin animals from the Tables I to III.

The effects of curare on the Hüerthle tracings may be reduced to the thre types shown in Figs. 1 to 3.

In type A (Fig. 1) the diastolic pressure falls considerably, the systoli pressure much less. The amplitude is somewhat increased.

In type B (Fig. 2) both the systolic and the diastolic pressures fall, usuall with a moderate decrease of amplitude.

TABLE IV.

INFLUENCE OF VAGI AND ATROPIN ON CURARE.

	Number of experiments.	Blood pressure (mm. Hg).			Curare Fall =		Secondary Rise =		Splenic flow slowed by (per cent).
		Before curare.	During curare.	After curare.	mm.	Per cent.	mm.	Per cent.	
Vagi intact	13	97	52	103	45	46	6	6	22
Vagi divided	15	121	67.5	127.5	53.5	44	6.5	5	13
Atropin, vagi intact .	7	88.5	69.5	106	19	22	17.5	20	14
All curare exp's . .	35	105.5	62	112	43.5	41	6.5	6	17

In type C (Fig. 3), the systolic pressure falls and the diastolic pressur rises. The amplitude is greatly decreased. Type A occurred in fifteen ani mals; B in seven, and C in only three animals. The heart, therefore, ap pears to be weakened in only three of the twenty-five animals.

All our data show that with the dose of curare used by us, *the bloo pressure and the vasomotor centre are practically normal within fiftee minutes after the injection* — at most, there is a slightly increased ton of the centre. We are therefore justified in transferring results obtained after this period to normal anæsthetized animals.

Comparison with previous work on curare. — The most extensive investigation of the circulatory action of curare is that of Tillie.[11] With small an medium doses (1 to 20 times "normal"), he also found the fall of blood pressure to be very short, with complete recovery in one-half to ten minutes Repetition of the injection again lowers the pressure. F. Pick,[12] using jus sufficient curare to paralyze the muscles, obtained on dogs with normal blood pressure (120 to 140 mm.), an average fall of 15 to 20 mm.; sometimes there was no fall. Our somewhat higher averages are probably due to more rapid injection. With very much larger doses (50 to 100 normal), Tillie found the fall to be more lasting and the recovery imperfect. With ordinary doses, the final pressure often rose considerably above the original in rabbits, but not in dogs or cats. In our experiments, dogs show a tendency to this secondary rise, but it is slight. Tillie also observed the curare fal' after division of the vagi and after atropin. To locate the mechanism of the fall, he excluded the vasomotor centre directly by destruction of the cord. We have shown that the centre is even somewhat stimulated. The fall must therefore be due to depressing the peripheral[13] vasomotor mechanism, or of the cardiac muscle. Tillie believed that the heart is not influenced even by the largest doses, but his published data are not satisfactory on this point. Our experiments show that even moderate doses occasionally depress the heart, presumably indirectly; but this is so rare as to be unimportant. The essential cause of the lowered blood pressure, therefore, lies in the peripheral vasomotor mechanism. Tillie also inclines to attribute the short duration of the vascular action to a transfer of the curare from the vessels to the skeletal muscles.

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[11] J. TILLIE: Archiv für. Experimentelle Pathologie und Pharmakologie, 1890 xxvii, p. 21.

[12] F. PICK: *Ibid.*, 1899, xlii, p. 410.

The effects of curare on the central vasomotor control of the kidney appear to be strictly analogous to its effects on the spleen. In three experiments the curare affected the kidney perfusion as follows: slightly slowed in one (C 30); negative in one (C 51); slightly quickened in one (C 82).

III. THE EFFECTS OF SCIATIC STIMULATION.

Stimulation of the central end of the sciatic nerve is a favorite method of testing vasomotor response. The beautiful quantitative studies of Porter on the pressure effects and the careful experiments of Pick and Burton-Opitz on the outflow are especially important in the present connection. Before discussing these, however, we shall give the results of our own experiments, since these furnish us with direct data on the vasomotor centre.

The effects of sciatic stimulation were tried mainly on anæsthetized and curarized dogs. As we have shown, the vasomotor system may be considered as being in a practically normal condition when th curare fall has passed off. The stimulations were with inductio currents and of such strength and duration (usually 1 to 5 cm. di tance of the Harvard coil for one minute) as to secure the maxima rise of blood pressure. Under these conditions, and with spleen pe fusions, the following results were obtained:

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[13] The word "peripheral" is applied by us exclusively to the extra-central vasomotor mechanism; and not, as is sometimes done, to the skin and extremities.

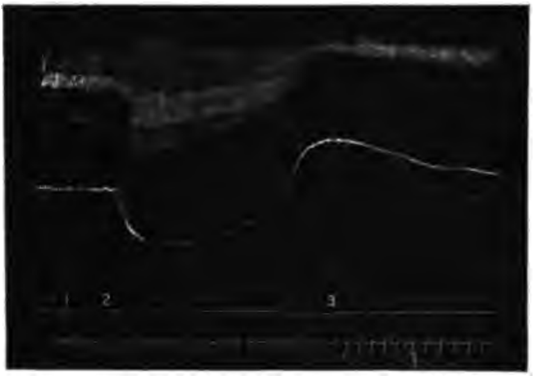

FIGURE 5. — *Typical Sciatic Response during Curare.* (Exp. C. 55–2.)

The average of thirt one such experimen show that sciatic stim lation raised the bloo pressure from 120 mn to 150 mm. with a r turn to 128 mm. aft the stimulation wa withdrawn. The activ rise, therefore, average 30 mm., or 25 per cen the residual rise (th level reached and mai tained several minut after stopping the stimulation) was 8 mm., or 6 per cent. Th Hüerthle tracings show increase of systolic, and especially of diastol pressure, so that the excursions appear somewhat diminished (Fig. 5

The spleen-flow was slowed by 20 per cent during the stimulatio and remained somewhat slowed (15 per cent) after the pressure ha returned to constant. The stimulation of the vasomotor centre, there fore, appears to outlast the actual stimulation of the sciatic (Fig. 4

The constriction is a very uniform phenomenon; but it is often precede by a quickened flow, due to the expression of the residual fluid by the co tracting spleen. (Conversely, the expansion of the spleen probably accoun in part for the slowness with which the flow recovers after the stimulatio is withdrawn.) Very exceptionally (in three cases out of thirty-two) th sciatic stimulation produced a pure quickening. One animal (C 10) showe all three reactions: the first stimulation produced a pure quickening; th second, pure slowing; the third, slowing preceded by quickening; the fourt pure slowing. The blood pressure rose with each stimulation.

Influence of division of both vagi on sciatic stimulation. — If th vagi have been divided, sciatic stimulation, on the average, raises th pressure somewhat more (10 mm.) and the vasomotor stimulation is also greater. The difference is not so large as to deserve much consideration. Division of depressor paths suggests itself as a possible explanation.

TABLE V.

INFLUENCE OF VAGI ON SCIATIC STIMULATION.

	Number of Experiments.	Blood pressure (mm.)		Rise =		Splenic flow slowed by (per cent).
		Before sciatic.	During sciatic.	mm.	Per cent.	
Vagi intact	10	121.4	145	23.6	19.5	9
Vagi divided	16	121.6	156.3	37.4	28.5	13
Atropin, vagi divided .	5	113.4	141.2	27.8	24.5	25

Influence of the level of the initial blood pressure on the sciatic stimulation. — In our experiments, the actual rise (in millimetres) was approximately the same for blood pressure ranging between 70 and 160 mm. The level to which the blood pressure rises, therefore, increases with the original pressure; and the percentile rise tends to vary inversely to the initial blood pressure. These results agree quantitatively with those of Porter. The residual rise is less for higher pressure, even when judged by millimetres. The degree of vasomotor stimulation, as expressed in slowing of the vein-flow, did not bear any simple relation to the blood pressure.

Comparison of sciatic stimulation with asphyxia. — For the sake of comparison, we may add that the vasomotor centre is stimulated much less by the sciatic than it is by asphyxia; in four experiments in which both measures were tested, the average slowing from sciatic stimulation was 19 per cent; from asphyxia 55 per cent, *i. e.*, about three times as great.

Comparison of the sciatic rise with that of aortic compression. — A comparison of the rise of blood pressure by sciatic stimulation with that produced by clamping the descending thoracic aorta may be of interest. In two experiments in which the two measures were compared, the pressure arose:

TABLE VI.

Influence of Blood Pressure on Sciatic Stimulation.[1]

Initial blood pressure. (mm.)	Number of experiments	Blood pressure (mm).			Rise in mm.		Percentile rise.		Slowing of spleen-flow. (%)	
		Before sciatic.	During sciatic.	After sciatic.	During sciatic.	After sciatic.	During sciatic.	After sciatic.	During sciatic.	After sciatic
70—103	10	93.5	125.7	—	32.2	—	34.5	—		
	7	90.3	121.9	97.6	31.6	7.3	35	8		
	6	90.0	122.0	98.0	32.0	8.0	35	9	20	11
105—120	8	118.4	147.3	—	28.9	—	25	—		
	5	115.4	146.0	132.6	30.6	17.2	26.5	15		
	3	117.0	157.0	137.0	40.0	20.0	34	17	20	16
130—140	8	135.1	162.4	—	27.3	—	20	—		
	7	135.9	161.9	141.6	26.0	5.7	19	4		
	3	135.0	162.0	146.0	27.0	11.0	20	8	9	5
140—165	5	152.6	186.2	—	33.6	—	22	—		
	4	149.5	177.8	149.8	28.3	0.3	19	0.2		
	3	151.0	177.0	151.0	26.0	0	17	0	56	34
Total Series	31	120.2	150.5	—	30.3	—	25.5	—		
	23	120.0	149.0	127.6	29.0	7.6	23	6	20	
	19	120.0	150.0	—	30.0	—	25	—		
	15	117.0	148.0	126	31.0	9.0	27	8	23.5	15

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[1] Complete data could not be obtained in some of the experiments, as is indicated in the table. Taking the "Total Series" as an example, the rise of blood pressure during stimulation was observed in 31 experiments. In 23 of these the residual pressure was also observed; in 19 the vein-flow was recorded, etc. All the available data were averaged separately for each of these subgroups. This had the further advantage of indicating the probable error in the averages. For instance, the rise of pressure for the 31 experiments was 30.3 mm. The subgroups of 23, 19, and 15 experiments all show practically the same rise; the chance of error is therefore very small. The residual rise is seen to be much more variable within each group; the chance of error is therefore much greater.

On sciatic stimulation, from 126 to 145 = 19 mm.

On aortic compression, from 129 to 204 = 75 mm.

The sciatic rise is therefore only a fourth of that produced by compression of the aorta.

The central vasomotor reaction of the kidney to sciatic stimulation. — This is in every respect similar to that of the spleen. In five stimulations (after curare) on three animals (C 30, 51, and 82) the kidney flow was slowed four times and was unaffected once. The average slowing amounts to 20 per cent, *i. e.*, approximately the same as in the spleen. (Burton-Opitz and Lucas[14] have pointed out that if the kidney is left connected with the systemic circulation, reflex renal constriction is more or less overcome mechanically by the increased blood pressure, so that the renal blood flow may be but slightly decreased.)

The central vasomotor reaction of the leg to sciatic stimulation. — This is also very similar to the spleen-reaction. It was observed in the following manner:

In dogs C 3 and 4, one leg was separated completely from the systemic circulation by ligating and dividing the muscles in small masses. The sciatic nerve was left intact. Perfusion was done through the femoral artery and vein, curare being injected both into the animal and into the perfusing fluid. The sciatic was stimulated on the opposite side. In five stimulations on these two animals, the flow was slowed three times, quickened once, and unaffected once.

In cats C 22 and 23, the perfusion was made through the iliac artery and vein, without dividing the muscles. The animals were curarized. The results were not so satisfactory; of four stimulations of the opposite sciatic, two were negative, one gave constriction, and one dilation.

In dog C 24, the perfusion was made from the femoral artery to the femoral vein without dividing the muscles. Stimulation of the opposite sciatic gave good slowing (25 per cent).

IV. Influence of the Curare Fall on Sciatic Stimulation.

As we have seen, curare causes a temporary fall of pressure by a peripheral vascular action. The effect of this on the response to sciatic stimulation opens up a series of very interesting problems.

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[14] Burton-Opitz and Lucas: Proceedings of the society of experimental biology and medicine, 1909, vi, p. 72.

We have therefore tabulated the results of sciatic stimulation three periods of the curare action: when the pressure was falli when it was recovering, and after recovery was complete. The res are shown in Table VII. (See also Table X.)

TABLE VII.

INFLUENCE OF CURARE ON SCIATIC RESPONSE.

Stage of curare.	No. of experiments.	Blood pressure.					Rise (mm).		Percent slowing spleen-fl	
		Initial.	After curare							
			Minimal.	Just prior to sciatic	During sciatic.	After sciatic.	During sciatic.	After sciatic.	During sciatic.	After
During fall of pressure .	14	111	56	56	89	107	33	51	31	
Incomplete recovery . .	4	106	52	81	122	120	41	39	*	
Pressure just recovered .	7	110	58	114	140	122	26	8	12	
Some time after recovery	31	120	—	120	150	128	30	8	20	

* Flow quickened by 15 per cent. ** Flow quickened by 30 per cent.

It appears that the pressure rises during (maximal) sciatic stir lation by the same number of millimetres, whether the curare act is present or absent (Figs. 4, 5, 6, and 7). The degree of the cur fall also had no influence on the sciatic response. In the experime in which the curare fall amounts to less than 30 mm., sciatic stir lation caused an average rise of 31 mm.; with the fall between and 70 mm., the sciatic rise averaged 33 mm.; with the curare over 80 mm., the rise also averaged 33 mm.

When the sciatic is stimulated during the fall, the rise does bring the pressure to the normal level; but almost invariably pressure continues to ascend when the stimulus is removed (Fig. This is due mainly to the spontaneous passing of the curare act (Fig. 7).

It may therefore be concluded that the peripheral vasomotor pression produced by curare does not lessen the degree of avera response to strong sciatic stimulation. In individual experimen however (for instance in C 30), it may be noted that sciatic stimu tion at the depth of the curare fall causes no rise, but resumes its efficiency a few minutes later. This suggests the possibility that the strength of the sciatic stimulation may influence the result. We have, therefore, made experiments in this direction:

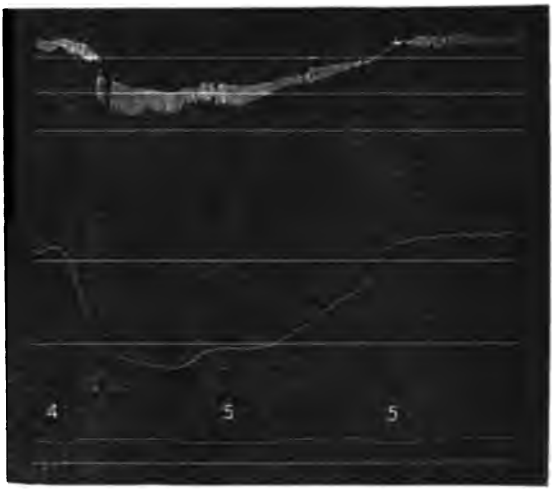

FIGURE 6. — *Sciatic Stimulation at Successive Stages of Curare.* (Exp. C. 31-1.) Note the continued ascent of the pressure after stimulation.

The influence of the intensity of sciatic stimulation on the reflex pressure response. — Porter and Richardson [15] have announced that the intensity of the response increases with the intensity of the stimulation, until a maximum is reached. Porter and Pratt [16] find that the same law applies to depressor stimulation. Our experiments on sciatic stimulation fully confirm these conclusions.

Using the Harvard coil with a gravity battery, the response to sciatic stimulation at various distances of the secondary coil was as follows:

Experiment C 84. Curarized dog with very slight respiratory response.

Serial number of the stimulation.	Distance of secondary coil.	Rise of blood pressure.	
12	10 cm. 70°	167–174	7 mm.
11	10 cm. 45°	170–185	15 mm.
14	10 cm. 45°	170–184	14 mm.
8	10 cm.	160–180	20 mm.
9	10 cm.	158–185	27 mm.
10	10 cm.	162–190	28 mm.
15	10 cm.	168–189	21 mm.
16	8 cm.	170–195	25 mm.
18	6 cm.	170–208	38 mm.
19	6 cm.	172–208	36 mm.

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[15] PORTER and RICHARDSON: This journal, 1908, xxxiv, p. 34.

[16] PORTER and PRATT: *Ibid.*, p. 35.

The influence of curare on sciatic stimulations of different intensity. – Non-curarized animals react so complexly to sciatic stimulatio that we abandoned the comparison of curarized and non-curarize animals, and instead compared the response before and after th vasomotor effects of the curare had worn off — using stimuli of diffe ent strength on the same animal.

The results, arranged in the order of the magnitude of respons after recovery, are briefly as follows:

Experiment number.	Distance of coils (cm.)	Rise of pressure in (mm.) on sciatic stimulation:		Level of blood pressure just before:		Reductio of the sci atic rise b curare.
		(a) After recovery from curare.	(b) During the vasomotor action of the curare.	(a)	(b)	
93	10	none	none	135	135	——
94	10	6	8	80	88	(—2)
90	10	8	none	192	200	8
94	7	17 12 } 14.5	10	80	80	4.5
82	10	20 15 } 17.5	8	105 140 }	77	9.5
90	6	22	15	185	200	7
84	10	27 28 } 27.5	20 21 } 20.5	158 162 }	160 168 }	7
94	0	32	32	78	90	0
93	0	40 35 } 37.5	34	125	128	3.5
90	0	43	32	182	120	11

If these data are averaged, it is found that, with:

Normal sciatic response of	The response under curare averages	The response is therefore reduced by
6–8 mm., average 7 mm.	4 mm.	3 mm. or 43 %
12–28 mm., average 20.4 mm.	13.2 mm.	7.2 mm. or 35 %
32–43 mm., average 37.5 mm.	33 mm.	4.5 mm. or 12 %

This shows that the curare, for a brief period after its injectio reduces very materially the response to sub-maximal sciatic stimul tion, while it interferes to a much less degree with maximal stimul tion. The curare fall of blood pressure can therefore be explained by peripheral blocking of the tonic vaso-constrictor impulses. With moderate doses of curare, this block is but partial and can be completely overcome by the stronger impulses resulting from maximal sciatic stimulations. With very large doses the block, according to Tillie (*l. c.* p. 29), is complete. Since barium still causes a rise, he concludes that the curare does not act directly upon the arterial muscle, but rather upon the nerve-endings. He made no attempt to exclude the vaso-motor ganglia. This point is still in need of investigation; but the prompt and fleeting action indicates rather strongly that Tillie's view is correct.

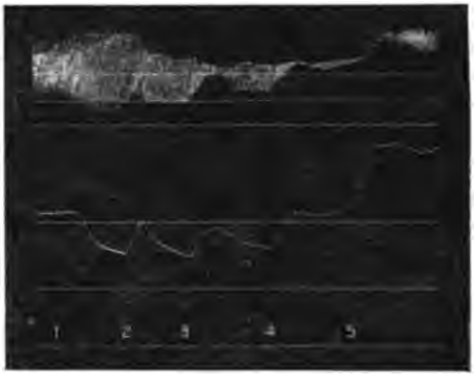

FIGURE 7. — *Sciatic Stimulation at Successive Stages of Curare.* (Exp. C. 26–1.) Note that the active rise is the same at 2, 3, and 4; the residual pressure, however, is higher after each stimulation, *i. e.*, as the curare action disappears. The high rise at 5 is asphyxial.

V. THE PERCENTILE AND ABSOLUTE RISE OF BLOOD PRESSURE AS CRITERIA OF VASOMOTOR RESPONSE.

The changes of blood pressure on stimulation of afferent nerves furnish the most convenient means for judging the excitability of the vasomotor centre. Systematic quantitative studies in this direction have been made by Porter and his pupils;[17] and our work, in so far as it covered the same ground, has yielded identical results. The interpretation of the results, however, is not always a simple matter. It is self-evident that the blood pressure ceases to be a reliable index of vasomotor response, if the cardiac activity or the total volume of blood are simultaneously altered. When such alterations can be ex-

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[17] These studies are conveniently summarized in PORTER's Harvey Lecture (Harvey Lectures, 1906, ii, p. 98). The originals, however, must be consulted for the quantitative data.

cluded, there still remains the fundamental question whether the a
solute or the percentile change in the blood pressure is the corr
index of vasomotor activity. This question becomes important wh
it is desired to compare the effects at different levels of blood pressu
as has been pointed out by Porter.[18] To use his example, "stimulati
of the sciatic nerve in the rabbit, while the blood pressure was 1
mm. Hg, caused a rise of 35 mm., and when the pressure was 50 mm
stimulus of equal intensity still caused a rise of 35 mm. The absolu
change was the same in both, but in the first instance this change w
35 per cent, while in the second it was 70 per cent." In other wor
if the absolute rise is used as the criterion, the vasomotor respon
would be judged to be the same at both pressures. If the percent
rise is the correct criterion, then the centre responded twice as pow
fully at the lower pressure.

It will be appreciated that the matter is of very considerable i
portance; for the use of the wrong criterion will lead to serious erro
in the interpretation of results. In the opinion of Porter (*l. c.*), t
proper criterion is the percentile change; but the analogy he adduc
in illustration of his choice is not convincing and might easily be us
to support the opposite argument: "An unfaithful trustee robs t
women. One of these has $40,000, the other $20,000. From each
takes $10,000. Their absolute loss is the same, but one woman c
still live on her income, while the other must work or beg."

With the same absolute loss, the percentile loss of the two wom
is different, it is true; but the percentile gain of the trustee is t
same in both cases. *A priori*, it is not clear whether the blood pr
sure is playing the role of the trustee or of the women. It is ve
evident that the problem must be approached in a more direct mann

Our method permits us to approach the question experimental
for it allows us to judge the reactions of the vasomotor centre at d
ferent levels of blood pressure, while the test object, the spleen, is n
exposed to the mechanical complications of altered blood supply.
we have seen, the absolute sciatic rise is practically independent
the blood pressure (unless the pressure is so low as to produce cereb
anemia and asphyxiation). The percentile rise is therefore the great
the lower the blood pressure. According to Porter's conception, t

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[18] W. T. PORTER: This journal, 1908, xxvi, p. 461.

TABLE VIII.

SCIATIC RESPONSE ARRANGED BY BLOOD PRESSURE.

Serial Number. (C)	Blood pressure before stimulation.	Rise in mm. during stimulation.	Slowing of spleen-flow (per cent).
14	70	25	30
42	87	18	25
43	90	48	18
15	93	52	13
1	100	40	24
11	100	10	11
43	112	23	36
33	115	15	0
1	120	40	20
13	120	30	—9[1]
39	120	35	25
25	120	55	5
36	120	13	—3[1]
16	130	20	18
29	135	45	12
10	138	22	—150[1]
41	140	15	0
10	145	23	50
10	148	15	57
37	160	40	65
29	165	55	18

[1] Flow quickened.

vasomotor centre reacts more powerfully the lower the blood pressure If this is true, then the slowing of the spleen-flow, by our method should be greater when the sciatic is stimulated at the lower pressures. Our actual data on this point are presented in Tables VIII IX, and X.

TABLE IX.

SCIATIC STIMULATION AT DIFFERENT PRESSURES ON THE SAME ANIMALS.

Serial Number (C).	Stimulation at lower pressures.			Stimulation at higher pressures.		
	Blood pressure before stimulation.	Rise in mm. during stimulation.	Slowing of spleen-flow (per cent).	Blood pressure before stimulation.	Rise in mm. during stim.	Slowing of spleen-flow (per cent).
1	100	40	22	120	40	20
29	135	45	13	165	55	17
36	105	25	—5	120	13	—3
43	90	48	18	112	13	42
Average	108	39	10	129	33	15

Table VIII shows the series of sciatic stimulations made after the curar effects have passed off. The experiments are arranged in the order of th blood pressure which pertained just before the sciatic was stimulated. Th significant figures are those in the last column, since the percentage reduction of the flow would indicate the degree of constrictor response. A glance wi suffice to show that the slowing does not decrease from the lower to th higher levels as it should do if Porter's assumption were correct. Th figures being taken from different animals, are naturally too irregular t yield reliable averages. It is necessary to eliminate the extremes. If thi is done,[19] the average slowing is somewhat less for the experiments below 120 mm. (equals 20 per cent) than for those above 120 mm. (equals 24. per cent). In other words, our direct results from this series speak agains Porter's assumption.

To eliminate the considerable chance of error introduced by the variabl response from different spleens, we present in Table IX the effects of sciati stimulation made at different levels on the same animals. Here it will be seen that the *constrictor response is uniformly somewhat greater at the high pressures*. This is even more than would appear from the figures; for at the higher levels the centre is already exerting somewhat more tone (the flow before stimulation averaging 16 per cent less than at the lower levels). The centre must therefore presumably send out even a somewhat more powerful impulse to obtain a further constriction.

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[19] That is, if experiments 33, 11, 43, and 14 are eliminated from those belo 120 mm.; and experiments 10, 41, 37, and 10 from those above 120 mm.

TABLE X.

SCIATIC STIMULATION AT DIFFERENT PRESSURES ON THE SAME ANIMALS UNDER CURARE.

Serial Number (C).	Stimulation at lower pressures.			Stimulation at higher pressures.		
	Blood pressure before stimulation.	Rise in mm. during stimulation.	Slowing of spleen-flow (per cent).	Blood pressure before stimulation.	Rise in mm. during stimulation.	Slowing of spleen-flow (per cent).
25	30	30	24	120	55	5
31	40	10	25	86	30	48
33	35	17	28	70	25	33
39	60	20	22	120	35	23
41	90	50	—8	140	15	0
41	60	10	8	80	6	—6
Average	53	22	14	103	27	18

Table X compares the response during the curare fall with that of the higher pressures, when the curare action had more or less disappeared. Although the difference of pressure is here very great (nearly 100 per cent), the response is still identical, or rather somewhat greater at the higher levels. (Here, again, the slow before stimulation averaged 15 per cent less than at the lower level.)

The facts elicited by our method therefore give a negative answer to Porter's assumption.

The vasomotor centre does not react more powerfully to sciatic stimulation at low pressures; and therefore the absolute rise is a more reliable criterion of its response than is the percentile rise.

We are inclined to believe that *a priori* reasoning is in entire acco with this experimental conclusion. The facts can be readily unde stood by assuming a simpler but strictly analogous example:

What will be the effect of two identical stimuli thrown into t same muscle at different times? Inevitably, if the muscle is in t same functional state, it will contract with the same force each tin Similarly, two stimuli of equal force, coming from the vasomot centre, will cause the arterial muscle to contract with equal forc (Again assuming for the sake of simplicity that the arterial musc is in the same functional state.) Let us say this force is equivale to 35 mm. of mercury, on the entire arterial system. If we have system containing fluid under pressure, and we allow an addition force of 35 mm. to act upon it, we shall increase the pressure by mm., no matter what the original pressure may have been. If th was 100 mm., it will be increased by 35 mm. or 35 per cent. If t original pressure was 50 mm., it will also be increased by 35 mm. not by 35 per cent, but by 70 per cent.

We have already seen that this is the actual fact, both in Porter tabulations and in our own. According to strictly physical laws, must expect that the same intensity of vasomotor stimulation wou raise the blood pressure by the same number of millimetres; and ther fore, that the percentile rise would be the greater, the lower the bloo pressure. In other words, the absolute rise and not the percenti rise, is the more eligible measure of the intensity of the vasomot stimulation.

We may remark at once that we do not conceive the conditions be quite as simple as we have sketched. For we are making the assum tion that the arterial muscle is always in the same functional stat no matter what the blood pressure — that it always responds to th same stimulus with the same force.

The remarkable agreements of the results with the theory seems show that this is the case within ordinary physiological limits; althoug it is probable that an artery which is stretched or relaxed beyond the limits would behave differently.

We do not know of any direct measurements of the influence of resistan on the extent and force of arterial contraction. However, the investigati of Asher shows that the change of volume of a limb with efferent vasoco strictor stimulation is the same whether the vessels were previously relax by heat or contracted by cold (within a range of temperature between 10.5 and 40° C.). "Für die Gefässmuskulatur der Wirbelthiere gilt nach meinen obigen Erfahrungen jedenfalls der Satz, dass hemmende und erregende Nerven gleich wirken, unabhängig davon, ob die Muskulatur relativ schlaff oder kontrahiert ist" (Zeitschrift für Biologie, lii, p. 310). This is in entire agreement with our conception. It is also well known that the shortening of skeletal muscle is not lessened by fairly high resistance (*e. g.*, Fig. 38, in Heinz, Handbuch der experimentelle Pathologie und Pharmakologie, i, p. 488).

Similarly, the pre-existing state of the vasomotor centre — whether or not it is already in a state of stimulation when the reflex stimulus is thrown in — may introduce complications. Of this our vein-flow observations offer some clear illustrations, as in Experiment C 29. This doubtless accounts for many of the irregularities.

Experiment C, 9 (*Chloroform*). — Before the administration of chloroform, sciatic stimulation raised the pressure from 165 to 220 mm. and slowed the spleen-flow from 73 to 60 units. Chloroform was then administered, and the pressure fell progressively. The fall was due entirely to cardiac weakening, for the spleen-flow was slowed, indicating (anemic) vasomotor stimulation. When the pressure had fallen to 120 mm., the sciatic was again stimulated. This caused a very powerful vasomotor stimulation, the flow being slowed from 63 to 32 units. Nevertheless, the blood pressure rose only 3 mm., the weakened heart evidently being unable to work against the high resistance.

As the pressure continued to fall, the asphyxial vasomotor stimulation became more and more intense, the spleen-flow being reduced to 7 units when the pressure reached 105 mm. At this point, sciatic stimulation caused no further slowing — not because the centre was paralyzed, but because it was already stimulated to its maximum response. (It is scarcely necessary to add that these results are not typical of the ordinary actions of chloroform.)

On the other hand, the blood pressure record may become totally unreliable, as an index of vasomotor stimulation if the cardiac force happens to alter at the same time as in the experiment just quoted. These irregularities, however, are not of sufficient frequency and extent to affect the averages.

We have so far considered only the pressor stimuli — but the case is precisely the same with depressor stimuli — for Porter has shown that the two obey the same laws (see especially the correspondence the curves on p. 404 of vol. 20, This journal). If we subtract a for of 35 mm. — or add a negative force of that magnitude — the pre sure in the system will be decreased by 35 mm. — again independent of the original pressure.

With this correction of the apparently erroneous assumption th the percentile change is the proper criterion of vasomotor respons some of the deductions which Porter had drawn must be revise although the revision is mainly quantitative. When he finds, f instance, that certain conditions or grades of shock and hemorrha with low blood pressure still give the same percentile response, th does not mean that the centre is unaltered; for since the absolu response is considerably less, the excitability of the centre must depressed. In another paper we shall show that this depression a tually occurs in moderate grades of hemorrhage. A fair degree activity remains, however, in the face of adverse conditions, so lo as these do not involve too severe and especially too prolonged anem or asphyxiation of the centre. The merit of this discovery of Porte is by no means diminished.

On the other hand, his deduction that the excitability increases the blood pressure falls must be abandoned. There is indeed an i crease of the automatic tone of the centre in all acutely induc anemias, as we shall show in another paper; but Porter's results well as ours show that this does not materially alter the reflex exci ability. As a protective mechanism, this increased automatic to would, of course, be vastly more useful than increased excitability unusual reflex stimulation.

Coming now to the observations of Porter and Richardson[20] on t vasomotor response of different animals the error of the percent method accounts very simply for the apparent anomaly of the do The results showed that the percentile sciatic rise averaged 47 p cent with all animals except the dog; in the dog it averaged only per cent. This the authors attribute to an idiosyncrasy for cura without advancing any evidence for their assumption. An examin tion of their tables, however, indicates that it is due simply to t fact that the average blood pressure of their dogs was 125 mm., wh the average of the other animals was from 38 to 88 mm.

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[20] PORTER and RICHARDSON: This journal, 1908, xxiii, p. 131.

Arranged by the absolute rise (and disregarding the cases in which the blood pressure was below 50 mm. and the vasomotor centre presumably injured), the animals would form the following series: —

Guinea pig	24–27 mm.
Rat	22–35 mm.
Dog	24–37 mm.
Rabbit	31–43 mm.
Hen	38–45 mm.
Cat	34–58 mm.

This criterion again leaves untouched the main fact, namely, that the vasomotor response of these different animals is remarkably similar, although not absolutely identical.

CONCLUSIONS.

1. A method is described for studying the reactions of the vasomotor centre, without the usual complicating factors.

2. The injection of curare slightly stimulates the vasomotor centre. The temporary fall of pressure is due to a peripheral action.

3. Maximal stimulation of the central stump of the sciatic nerve stimulates the vasomotor centre so as to decrease the spleen-flow by 20 per cent, on an average (the blood pressure rising 30 mm. or 25 per cent). The result is not affected by previous section of the vagi.

The absolute rise of the pressure is the same for blood pressures ranging between 70 and 160 mm. The percentile rise therefor varies inversely to the level of the blood pressure. The response of the vasomotor centre does not bear any simple relation to the blood pressure.

The response of the vasomotor centre to sciatic stimulation is only about one-third as powerful as is its response to asphyxia.

4. During the brief vasomotor action of curare, there is a considerable peripheral block to weak and moderate vasomotor impulses. This may be completely overcome by strong vasomotor stimulation (if the quantity of curare is not too large).

5. The absolute change of the blood pressure appears to be a more correct index of degree of vasomotor response than the percentile change.

ACAPNIA AND SHOCK.[1]—VI. ACAPNIA AS A FACTO IN THE DANGERS OF ANÆSTHESIA.

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[From the Physiological Laboratory of the Yale Medical School.]

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I. EXCLUSION OF AFFERENT IRRITATIONS BY ANÆSTHESIA.

ONE of the strongest points in favor of the acapnia theory o shock is afforded by the facts of anæsthesia. Chloroform, ether morphin, and other narcotics and anæsthetics tend to prevent shock They likewise prevent, or considerably diminish, the excessive respiration of pain. They raise the afferent threshold of the respiratory centre to such an extent that all but the most intense irritations ar excluded. According to our view this irresponsiveness of the respiratory centre in anæsthesia, and the consequent absence of excessiv breathing, obviate more or less completely the development of acapnia For this reason, and to the same extent, anæsthesia obviates shock.

The most striking contrast between the behavior of an anæsthe tized man under a major operation nowadays, and that of a man under a like operation before the discovery of anæsthetics, is afforded by their breathing. Formerly every stroke of the surgeon's knife elicited the rapid respiration of pain. Now the patient breathes deeply, slowly, quietly. It is probable that in the prevention of shock by anæsthetics, the abolition of consciousness is merely incidental. It is the influence of intense afferent irritations upon the centres of the spinal bulb, not upon those of the cerebrum, which induces shock. Death from sheer pain shortly after surgical operations was formerly a frequent occurrence. Such fatalities were probably of the type described in the preceding paper of this series.[2] It was there shown that fatal apnœa vera is the natural consequence of prolonged pain-hyperpnœa.

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[1] This series of papers originated in the observation (upon dogs) that limitin the rate of pulmonary ventilation prevents shock. This journal, 1908, xx p. 126. A confirmation of this fact upon 100 surgical patients, including fift laparotomies, in the Johns Hopkins Hospital has been published recently by W D. GATCH, Journal of the American Medical Association, 1910, liv, p. 775.

Nevertheless deaths still occur on the operating table, and in the majority of cases respiration fails first. These fatalities are now, however, supposed to be caused by the anæsthetic itself. They are explained vaguely as due to the condition of the subject. Often they are charged to the lack of skill of the anæsthetist. Yet the most skillful anæsthetist has to rely wholly upon empiricism and art. There is at present no clear scientific comprehension of the reason why, under apparently similar conditions, and with the same quantity of anæsthetic, one case continues to breathe regularly, while another passes into apnœa.

II. FAILURE OF RESPIRATION UNDER ANÆSTHESIA.

The highest percentage of mortality of any series of anæsthesias of which we have knowledge occurred in this laboratory three years ago. The subjects were thirty cats, all more or less wild. The anæsthetists were students without previous experience. The anæsthetic was chloroform. Most of the animals resisted anæsthesia; they struggled and breathed vigorously. Within twenty minutes after the anæsthetists took charge fifteen of the animals had stopped breathing. Ten of them died. Five others were only saved by persistent artificial respiration. In thirteen cases the apnœa occurred before the animal had been touched by a knife.

Consideration of the course of events in these cases brought out clearly the point that the animals which breathed excessively before anæsthesia were those which most readily ceased to breathe after anæsthesia. During mental excitement they performed forced breathing, and induced in themselves acapnia. When anæsthesia was induced, the flow of excitement from the cerebrum to the respiratory centre was stopped, and they sank into apnœa vera. In most cases the duration of hyperpnœa and degree of acapnia were probably insufficient to produce apnœa of fatal duration, except for the coöperation of another factor. Collingwood and Buswell[3] have shown that chloroform raises the threshold of the respiratory centre for CO_2. In other words, more than the ordinary quantity of CO_2 in the blood is needed to keep the respiratory centre active during deep chloroform anæsthesia. Thus spontaneous breathing could not recommence until the blood and tissues of the animals had accumulated even more than the quantity of CO_2 which they had previously lost. ***The rise of the threshold plus the depth of acapnia together determine the duration of apnœa.*** In a few cases the anæsthetists were careless and poured the chloroform on to the vaporizing masks in such quantity as to produce immediate paralysis of respiration and heart. But in the majority of the fatalities under our observation, even with unpracticed student anæsthetists using so potent an anæsthetic as chloroform upon so susceptible a subject as the cat, the death of the animal was not properly attributable to carelessness.

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[2] Y. HENDERSON: This journal, 1910, xxv, pp. 399–401.

The mishaps in these cases were due to the fact that pharmacology[4] has not applied to the conditions of anæsthesia the *capnial* (*i. e.*, CO_2) theory of respiratory regulation, — as developed by Traube, Miescher, Zuntz, Mosso, Fredericq, and especially by Haldane and his collaborators. We believe that the same explanation will apply to the majority of the fatalities under anæsthesia in man. Skill in administering anæsthesia consists mainly in avoiding acapnia by keeping the threshold of the respiratory centre for CO_2 at a uniform level. The ideal would be never to allow it to vary from the normal. The unpracticed anæsthetist — at least among our students when learning to operate upon animals — administers the vapor of ether or chloroform irregularly. He brings the subject under slowly and with hesitation. He thus prolongs the stage of excitement in which the threshold is subnormal and respiration excessive. Later he supplies at times so little anæsthetic that the subject again develops hyperpnœa, and the degree of acapnia is thus intensified. If he then "crowds on the anæsthetic," apnœa inevitably results. Even if, heedful of warning, he administers no more than is necessary for light anæsthesia, the respiration may fail. ***A subject, which has breathed regularly under deep anæsthesia at the beginning of an operation will cease breathing under light anæsthesia after it has become acapnic.*** Apnœa follows a sudden elevation of the threshold of the respiratory centre by increase of anæsthesia. The new level need not be above normal, — it may even be much below that of ordinary life, — yet if the CO_2 content of the body has previously been reduced far below this level, apnœa of fatal duration may occur.

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[3] COLLINGWOOD and BUSWELL: Proceedings of the Physiological Society, Journal of physiology, 1907, xxxv, p. 34, and xxxvi, p. 24.

[4] See the review by R. MAGNUS: Ergebnisse der Physiologie, 1902, i, 2, p. 40

The liability to acapnia is much greater when anæsthesia is induced with ether than when it is initiated with chloroform. This is especially true of dogs. Ether is generally regarded as a safer anæsthetic than chloroform. In reality the difference between their risks lies mainly in this: The results of unskillfulness with chloroform are immediate and are therefore easily recognized. The penalties for careless etherization on the contrary are deferred, and thus fail of recognition. If one intends to kill a dog, it is easy to do so with chloroform. For a careful anæsthetist, however, there is little risk in initiating anæsthesia with chloroform. The dangers are obvious; the period of excitement is brief. With ether administered by an "open cone" or "drop" method, we have found it practically impossible to induce failure of respiration in dogs which were not acapnic. Ether is a weaker anæsthetic for dogs than for the majority of human subjects, — unless they be habitual alcoholics. ***Nevertheless, to begin with ether (in dogs without morphin) is to invite ultimate disaster.*** The period of excitement is prolonged; a considerable degree of acapnia develops; and even after anæsthesia is attained the respiration continues to be excessive. Then suddenly the animal ceases to breathe, and, unless restorative measures are immediate and persistent, death results. Before we had recognized its causal relations, this series of events was the commonest accident in this laboratory. We know of another laboratory (outside New Haven) in which five mishaps of this sort occurred recently at the hands of an experienced investigator within

the short period of two weeks. The following cases afford illustration
of such fatalities in our hands:—

A strong young Irish terrier of about twelve kilos weight had been give
to us (Nov. 15, 1905), because it had developed an irritable temper. Th
experiment which we had planned needed as nearly normal respiration a
possible. We supposed (erroneously) that this object would be best a
tained without morphin. We attempted to bring the animal under ethe
It fought, breathed excessively, and then ceased breathing. After the re
piration was restored it again breathed excessively. Finally, just after th
animal had become quiet enough to place on the table, we found that
had again stopped breathing, although only a minute before it was so lightl
anæsthetized that its eyes winked. Traction on the tongue, sensory stimu
lation, and artificial respiration by compression of the throax failed to i
duce a return of breathing. A few minutes later the heart failed.

A medium sized dog (9.0 kilos) received subcutaneously half a gra
(0.003 gm. per kilo) of morphin sulphate. Fifteen minutes later it wa
etherized without more than ordinary resistance. On the table it develope
a rapid respiration, breathing thirty-five to forty times a minute for a
hour and a quarter. Then it stopped quite suddenly and forever, — i
spite of all the ordinary restorative measures.

It often happens that a dog which has passed through prolonge
ether-excitement becomes practically uncontrollable and inoperabl
At one moment it is nearly out of anæsthesia. At the next it ceas
to breathe after a few inspirations of weak ether vapor. After eac
period of apnœa the tendency to hyperpnœa becomes intensified, an
vice versa. This increasingly unbalanced condition of the respirato
centre may be due in part to an accumulation of the products
asphyxial acidosis (*e. g.*, acetone)[5] in the blood during the periods
apnœa, and the rapid oxidation of these substances during hyperpnœ
The alternations of depression and excitement are less regular b
more intense than those occurring in Cheyne-Stokes breathin
Pembrey[6] has shown that periodic respiration is relieved by admini
tration of oxygen or by CO_2 in proper dilution, and even better b

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[5] On the occurrence of acetone in the urine after anæsthesia see BALDWIN, H
Journal of biological chemistry, 1906, i, p.239.

[6] PEMBREY, M. S.: Journal of pathology and bacteriology, 1908, xii, p. 2
(bibliography). See also FILEHNE and KIONKA: Archiv für die gesammte Phy
ologie, 1896, lxii, p. 233.

oxygen containing a small percentage of CO_2. During recovery from prolonged etherization human subjects often breathe subnormally and become cyanotic. This behavior is probably due to acapnia, and tends to induce anoxhæmia and asphyxial acidosis. Hence the benefits of oxygen. In preceding papers of this series [7] dealing with dogs under similar conditions, it was demonstrated that small amounts of CO_2 restore a normal amplitude of breathing. Thus anoxhæmia was obviated.

Thus, although ether and chloroform have (in dogs) almost diametrically opposite effects upon the respiratory centre, yet both may induce fatal apnœa vera, — one indirectly, the other directly. For an acapnic subject ether might be described as a "powerful respiratory depressant," although it probably never (in dogs) elevates the threshold for CO' above the normal. Light or moderate etherization induces excessive breathing; a subsequent profound etherization involves a compensatory subnormal breathing. The *capnicity* of the subject at the time determines whether a certain degree of etherization shall act as a stimulant or as a depressant. We have observed the occurrence both in men and in dogs of marked individual variations in susceptibility to ether-excitement. Different qualities of ether, and even the best grades, appear to vary in producing hyperpnœa.

For laboratory purposes the dangers of acapnia are obviated in dogs by the following procedures: Morphin sulphate in the dosage of 0.010 to 0.015 gm. per kilo body weight is given subcutaneously. Half or three quarters of an hour later anæsthesia is rapidly induced with chloroform. Thereafter ether alone is used, except when the respiration and pulse begin to accelerate. A small amount of chloroform, if given promptly when this tendency develops, controls it without much danger of prolonged apnœa. The reason for the delay after morphinization lies in the probable fact (as it appears to us, although we have no quantitative observations upon it) that at first, during the stage of nausea, respiration is augmented by morphin. The advantages of beginning with chloroform are similar to those for which nitrous oxide is employed in initiating anæsthesia clinically.

The danger involved in administering chloroform to an acapnic subject may be illustrated by the following case: A child had swallowed an open "safety" pin. During the passage of the pin through the alimentary canal there was considerable suffering. Finally, durin defecation the pin caught in the anus. The efforts of a surgeon t remove it were unsuccessful, but caused the child much pain accom panied by hyperpnœa. Chloroform was administered, and the anu was dilated. As usual during the latter procedure, the patient dre several deep breaths. Then respiration ceased, and it was only afte half an hour of artificial respiration that spontaneous breathing wa restored. The anæsthetist, who told us of the case, is a careful ma and blames himself for what might have been a fatal accident. W however, believe it to be impossible for any one, no matter how carefu to administer chloroform by present methods to a subject rendere acapnic by days of discomfort and an hour of pain, without grea risk of apnœa.

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[7] This journal, 1910, xxv, pp. 314, 328, 388, and 392.

Forty years ago Hermann Fischer[8] wrote of sudden death under surgic operations: "Früher vor Anwendung des Anästhesirens war dieses Ereigni wohl häufiger. . . . Heut zu Tage pflegt man dergleichen Ereignisse unt Seufzen und Kopfschütteln dem Chloroform zuzuschreiben. . . . Man thu also diesem unvergleichlichen Mittel schweres Unrecht, wenn man ihm al Todesfälle zur Last legt, die während der Narkose sich ereignen. Sie wisse dass der Chloroformtod in einer Reihe von Fällen unter Erscheinungen de Asphyxie — die Athmung hört auf, das Herz schlägt noch eine Zeit lan weiter, — in einer zweiten unter dem Bilde der Herzlähmung — das Her steht plötzlich still, die Respiration geht noch eine Zeit lang weiter — ein tritt. . . . Wenn das Chloroform so giftige Wirkungen äussern könnte, we shalb tretten dieselben so selten ein? *Grosse Dosen werden wiederholt m Leichtigkeit vertragen und kleine Gaben führen wieder jäh den Tod herbei Das ist noch von keinem Gifte bekannt.* . . . Sie werden aus dem, was ic Ihnen über den Chloroformtod gesagt habe, leicht ermessen können, wi gefährlich die Anwendung des Chloroforms im Shok ist. . . . Man sc solche Patienten zuvörderst nicht chloroformiren."

III. THE THRESHOLDS OF THE RESPIRATORY CENTRE.

It is advantageous for the purposes of pharmacological analysis t differentiate between the threshold of the respiratory centre for affe ent influences, and its threshold for CO₂. This distinction corre sponds to a real difference, although the term "threshold" is merel a symbol for an unknown mechanism. The distinction may be illustrated by the action of morphin, chloroform, and ether. A dog which has received 0.03 gm. of morphin sulphate per kilo body weight becomes comatose. Its breathing is diminished until the heart beats only about 60 times a minute or even less. This pulse rate indicates a CO_2 content in the arterial blood of 50 volumes per cent or more — *i. e.*, one fourth or more above normal. This hypercapnia is an index of a corresponding rise in the threshold of the respiratory centre for CO_2. Loewy[9] has demonstrated such a rise in man under morphin. The afferent threshold of the centre is also raised, but relatively much less than the CO_2 threshold. If the animal is disturbed not only does its breathing alter, but it may even crawl away from the irritation, although the stimulus is of less than painful intensity. Similarly, a man who has taken an overdose of morphin may die of respiratory inactivity if left to himself, because of the rise of the threshold for CO. But if the narcosis is not too deep he may be kept breathing by afferent irritation.

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[8] Fischer, H.: Ueber den Shok, Volkmann's Sammlung klinischer Vorträg 1870, no. 10, pp. 10, 11, and 12. The italics are ours.

On the other hand, chloroform raises the afferent threshold of the respiratory centre more readily and to a relatively greater extent than it does the threshold for CO_2. A dog in light or moderate chloroform anæsthesia responds to no irritation of less than painful intensity. Brief but otherwise painful surgical operations upon human subjects are constantly performed painlessly and without increased breathing under very light anæsthesia. As will be seen from the blood gas analyses tabulated in the fifth section (p. 274), animals under such anæsthesia often have no more than the normal content of CO_2 in the arterial blood. In other words, the threshold of the centre for CO_2 is altered very slightly while the afferent threshold is greatly elevated.

Ether is often loosely described as a "respiratory stimulant." This expression, when translated into the terminology here employed, signifies that in a subject under ether the threshold for CO_2 may be below normal. At the same time the afferent threshold may be elevated sufficiently for surgical operation. As was shown in the two preceding papers of this series, the respiratory centre of a dog under ether without morphin usually maintains a more or less excessive pulmonary ventilation. Yet in this condition of the animal the breathing is not affected by afferent irritations of such strength as are produced by a cut through the skin. The CO_2 threshold is sub-normal, and the afferent threshold super-normal.

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[9] LOEWY, A.: Archiv für die gesammte Physiologie, 1890, xlvii, p. 601.

Broadly stated, those drugs which, if administered before pain, tend to prevent the development of acapnia and shock, will increase the tendency to failure of respiration, if given after acapnia has developed. The influence of ether in moderate amounts upon acapnic subjects sometimes affords apparent, but only temporary, exceptions to this statement. The difference between an anæsthetic which is a "respiratory depressant" and one which is a "respiratory stimulant" may be illustrated as follows: When an acapnic subject is chloroformed, apnœa may develop before the corneal reflex is abolished. The behavior of the respiratory centre may fitly be compared to that of a debtor upon whom a mortgage is foreclosed. On the contrary, when such a subject is etherized, the breathing may continue and may even become hyperpnœic. The behavior of the centre in this case resembles that of a man who is insolvent, but who escapes immediate bankruptcy by borrowing the money with which to pay interest on his debt Apnœa is temporarily avoided at the expense of an increase in the intensity of acapnia. ***Sooner or later every molecule of CO_2 lost from the body's reserve store must be replaced if the subject is to return to normal life.***

Among the signs watched by anæsthetists are the size and responsiveness of the pupils of the eyes. Acapnia induces in dogs an extreme dilatation Conversely, Guthrie, Guthrie, and Ryan find that in all animals the first stage of asphyxia causes a marked constriction. It is probable, therefore that the influences of morphin, ether, and chloroform upon the pupil, as upon the heart-rate, are mainly secondary to alterations in the blood gases.[10]

IV. THE RELATION OF THE THRESHOLD FOR CO_2 TO THE QUANTITY OF CO_2 IN THE BODY.[11]

Failure of respiration under anæsthesia is not the simple and direct toxic effect of an excessive dosage that it appears. It is rather the resultant of two (or more) factors, — the level of the threshold and the quantity of CO_2 in the body. The former is determined chiefly by the degree of anæsthesia at the time. The latter depends upon the balance between CO_2 production and elimination during the period, or periods, preceding.

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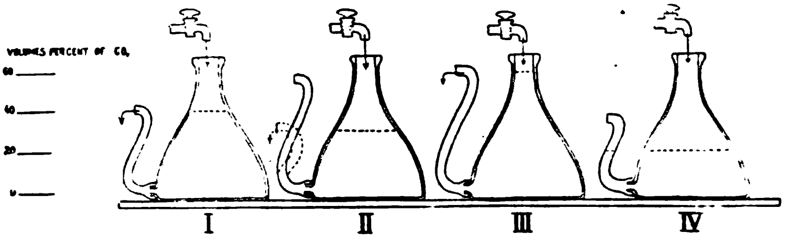
[10] Guthrie, Guthrie, and Ryan: Science, 1910, xxxi, p. 395. See also firs paper of this series, This journal, 1908, xxi, p. 126.

[11] A preliminary statement of this topic was presented by us before the American Physiological Society, Dec., 1909. This journal, 1910, xxv, p. 12.



FIGURES 1, 2, 3, and 4. — The jar here represented is assumed to have a capacity of 30,000 c.c. when filled to the level of 40 on the scale at the left. Under all the conditions here considered a stream of water of 300 c.c. per minute runs in from the stopcock. These quantities represent the production of CO_2 in the body at rest and the store normally held by the blood and tissues. The rubber tube at the side of the jar affords an outlet of such size that, when the free end is held far below the level of the water in the jar, one half its contents would run off in ten or fifteen minutes. This excessive drainage, occurring when the threshold at the open end of the tube is lowered, represents hyperpnœa. When the threshold of the tube is lifted above the level of the contents of the jar and the outflow ceases until water accumulates up to the higher threshold, an analogy for apnœa is afforded. Fig. 1 represents normal quiet breathing. The end of the tube and the contents of the jar are both at the normal level, and inflow and outflow (*i. e.*, CO_2 production and elimination) are equal. In Fig. 2 a period of hyperpnœa (represented by the tube in the position of the broken lines) has diminished the contents of the jar, and the threshold has then suddenly been elevated even above the normal level. The consequent prolonged cessation of outflow corresponds to the failure of respiration under chloroform. In Fig. 3 both the threshold and the contents of the jar are supernormal. The outflow through the tube represents the spontaneous breathing of a subject in profound morphin chloroform anæsthesia. In Fig. 4 the threshold is at a normal level, but outflow does not occur because the contents of the jar have previously been diminished. This represents the apnœa in a normal man after forced breathing. It shows also the failure of respiration in an acapnic subject when full ether anæsthesia is induced. Much the same conditions occur during the return to consciousness after prolonged anæsthesia under ether without morphin.

The relations of these factors are expressed graphically in Figs. 1 to 4. Here a glass jar represents the living body. The production of 300 c.c. of CO_2 per minute in a man at rest is typified by a stream of water of this volume and rate continually flowing into the jar. The storage capacity of the human body for CO_2 is not exactly known, but in the figures it is assumed that, if no outflow at all occurred, the stream would need to flow in for one hundred minutes in order to fill the jar from entire emptiness up to the level 40. This level represents the normal quantity of CO_2 in the body,—the quantity at which the arterial blood contains 40 volumes per cent of this gas.[22] The level of the threshold of the respiratory centre for CO_2 is represented by the height at which is held the end of the rubber tube which furnishes the outlet for the jar. When the outlet is held below the level of the water in the jar, the rapid outflow of water affords an analogy to the hyperpnoea occurring when the threshold of the centre is depressed below the quantity of CO_2 in the arterial blood. When the end of the tube is raised above the level of the water in the jar, the outflow will cease until the supply accumulates up to the height of the threshold. This cessation of outflow is analogous to apnoea. In Fig. 1 the ordinary conditions of breathing are typified. Both threshold and quantity are at the normal level and the inflow and outflow are equal. In Fig. 2 is represented the chloroform apnoea of the cats and child previously mentioned. Here the outlet tube has been held down as shown by the dotted line until the water in the jar has sunk to a low level. Then the tube has been raised suddenly to a height considerably above the normal. Evidently there can be no outflow through the tube until the store accumulates up to this high level. Similarly, apnoea in the cats and child lasted until CO_2 accumulated up to the supernormal level to which deep chloroform anaesthesia lifts the threshold of the respiratory centre. In Fig. 3 is expressed the condition of spontaneous breathing in deep morphia-chloroform anaesthesia when both the threshold and the quantity of CO_2 are far above normal. In Fig. 4 is represented the apnoea induced by full ether anaesthesia with the threshold [illegible] restored to the normal level after prolonged [illegible]. This diagram is especially interesting because it also shows the conditions to which nearly every subject who has been [illegible] for a length of time is exposed during the return to consciousness.

The foregoing discussion and its implications may be summarized [illegible] under [illegible] [illegible] and in other conditions, the degree of activity or inactivity of respiration at any time is the resultant of five sets of factors:

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[22] [illegible] is really only a half [illegible] will not be [illegible] Henderson [illegible]. This [illegible]

(1) The threshold of the centre for CO_2. — This threshold is elevated by morphin, by chloroform, and probably in man by deep ether anæsthesia. In dogs deep etherization (without morphin) merely restores a nearly normal level. The threshold is lowered in dogs, and probably in alcoholic and other excitable human subjects, by light etherization. The acidosis substances resulting from anoxhæmia have a similar lowering effect. Fear also appears to depress this threshold.

(2) The CO_2 content of the blood. — This quantity depends upon the relative rates of production and elimination of CO_2 during the period preceding, and upon the retaining capacity (*i. e.*, protein-alkali-acid equilibrium) of the tissues and fluids of the body.

The tension of CO_2 in the blood to the respiratory centre rather than the quantity is usually supposed to be the regulatory influence. Doubtless this view is correct, but we are unwilling to exceed our own data. Our blood gas analyses show only the quantity, — not the tension. It is often assumed — but in our opinion without sufficient evidence — that a condition of acidosis greatly diminishes the capacity of the blood to hold CO_2. Thus it is supposed by some writers that in acidosis the quantity of CO_2 would be lessened, while the tension might be normal. We do not believe [13] that (at least in respect to respiration) "acidosis" is "acid intoxication." In our opinion the acidosis substances influence the centre in the same manner as does ether. Beddard, Pembrey, and Spriggs [14] have shown that in diabetic coma with acute acidosis and intense acapnia the blood is capable of holding large quantities of CO_2. No one has yet reported simultaneous determinations of CO_2 quantity and CO_2 tension in such blood. In diabetic coma both the tension of CO_2 in the alveolar air of the lungs and the quantity (volumes per cent) of CO_2 in the blood are greatly diminished.

(3) The threshold of the centre for afferent nervous influences. — This threshold is raised by acapnia, by morphin, by chloroform, and by ether in all quantities. It is lowered by fear.

(4) The intensity and volume of the stream of painful nervous

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[13] *Cf.* Henderson, Y.: Proceedings of American Physiological Society. This journal, 1910, xxv, p. xii.

[14] Beddard, Pembrey, and Spriggs: Proceedings of the Physiological Society, Journal of physiology, 1909, xxxi, p. 44 (also Lancet, May 16, 1903).

impulses from any locus of irritation. — The flow of excitement from the cerebrum to the respiratory centre during mental anguish induces hyperpnœa (weeping) in essentially the same manner as does intense irritation of afferent nerves and sense organs. Pleasurable sensations and emotions also induce hyperpnœa (laughter), but with the exception of hysterical persons the effects are immediately and automatically compensated.

3. The inhibitory influence of the cerebrum upon respiration. — This is the element in breathing which is called "fortitude" or "physical courage," or the "ability to withstand pain."

Figurative language is necessary in a discussion of this sort. It is important, however, to distinguish figure from fact. Even the term "respiratory centre" is of course merely a figure, although some writers refer to "nerve centres" as if they were definitely known realities. The terminology of Haldane, which we have used in this paper, speaks of the "respiratory centre" as being "stimulated by CO_2" when the tension of this substance in the blood is "above the threshold value." This view of respiration is in accord with the present fashion of "hormone regulation," and is extraordinarily serviceable now. It is not improbable, however, that sooner or later there may be a revival of the opposite idea — that respiratory activity is essentially reflex. In recent years Sherrington[1] has introduced a substitute for "nerve centre" in the beautifully analytical conception of the "synapse." In this terminology the facts of chemical influence upon respiration may be expressed thus: Carbon dioxid, ether and the acidosis substances diminish, while morphin and chloroform increase, the resistance of the respiratory synapses to the passage of the excited state from the afferent to the efferent neurones. Ether produces anæsthesia, but has little influence upon the passage of the final common path (e.g., the phrenics, etc.) by the [illegible] (e.g., the vagi, etc.). Morphin tends to block the flow of excitation into the efferent channels both from the automatic afferent paths and from pain paths, but unlike ether it affects the former more readily than the latter. Such language would express the differences between the [illegible] of pain and the diaphragmatic activity of hyperpnœa more readily than does the phraseology which we have used. The latter takes account only of the [illegible] pulmonary ventilation. Furthermore, it is [illegible] experiment[2] and from

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[1] Sherrington, C. S.: The integrative action of the nervous system, 1906, pp. [illegible]

[2] [illegible] des Nervensystems, [illegible]

Sherrington's analysis of the spinal reflexes that a ***chemical influence upon respiration is not effected by an alteration in the metabolism of nerve cell bodies (perikarya), but is due to a change in permeability to excitation in the (theoretical) dendritic membrane of the synapse.*** The function of perikarya is merely nutritive. Thus lack of oxygen paralyzes neurones by starving their cell bodies, while on the contrary the acidosis substances resulting from partial asphyxia of other tissues are stimulants at synapses. We are doubtful of the idea that a formation of lactic acid in the respiratory centre itself, as suggested by Douglas and Haldane,[17] is the explanation of hyperpnœa from deficiency of oxygen.

V. VARIATIONS IN THE THRESHOLD FOR CO_2 IN ANÆSTHESIA.

In Table I are collected the results of analyses of the gases of the arterial blood of forty-five dogs under various forms and degrees of anæsthesia. From each of these animals many samples of blood were taken for analysis in the various lines of experimentation which have been, and are to be, described in this series of papers. We have reproduced here, however, only those data which were obtained before the animal had been subjected to artificial respiration, pain-hyperpnœa, acidosis, exposure of viscera, or other condition which would alter the threshold or quantity of CO_2. The alterations from the normal here shown were almost wholly due to the conditions of anæsthesia. All of the subjects were breathing spontaneously at the time the samples of blood were drawn. The figures for the CO_2 content of the blood afford therefore an approximate expression of the level of the threshold of the respiratory centre at these times.

In the normal life of dogs the centre probably regulates the pulmonary ventilation so as to maintain about 40 volumes per cent of dissociable CO_2 in the arterial blood. At the top of the third column of the table we find 63 volumes per cent under deep chloroform-morphin anæsthesia, and at the bottom only 23 volumes per cent under ether, *i. e.*, in ether-excitement, without morphin. It would certainly not be difficult to obtain figures both higher and lower than the extremes here shown, but even in the table as it stands a variation in the level of the threshold from a half above, down to a half below normal is demonstrated. Near the middle of the table a large number

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[17] DOUGLAS and HALDANE: Journal of physiology, 1909, xxxviii, pp. 406 and 407.

TABLE I.

Arterial Blood Gases from Forty-five Dogs Showing the Variations in [illegible] and Degrees

Number of exp.	Arterial blood gases, volumes per cent. O_2	Arterial blood gases, volumes per cent. CO_2	Morph. sulph. gms. per kilo.	Chloroform.	Ether.	Pulse per min.	Respiration per min.
1	30.3	63.1	.005	II	..	40	[illegible]
2	18.4	56.3	.007	III	..	51	[illegible]
3	13.8	50.4	..	III	..		
4	20.2	48.4	.07	[illegible]	.	60	[illegible]
5	[illegible]	48.5	[illegible]	..	[illegible]		
6	[illegible]	48.2	[illegible]	..	[illegible]		
7	[illegible]	[illegible]	[illegible]	..	[illegible]		
8	[illegible]	40.8	[illegible]	..	[illegible]		
9	[illegible]	[illegible]	.05	[illegible]		[illegible]	[illegible]
10	[illegible]	[illegible]	.01	[illegible]			
[illegible]	[illegible]	[illegible]	.01	.	I		
[illegible]	[illegible]	[illegible]	.01		I		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	
[illegible]	[illegible]	[illegible]		[illegible]			
[illegible]	[illegible]	[illegible]	[illegible]		I		
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	
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[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	
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[illegible]	[illegible]	[illegible]	[illegible]	.	[illegible]		
[illegible]	[illegible]	[illegible]	.	[illegible]		[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]				

TABLE I.

THE THRESHOLD OF THE RESPIRATORY CENTRE FOR CO_2 UNDER VARIOUS FORMS OF ANÆSTHESIA.

Number of exp.	Arterial blood-gases, volumes per cent.		Morph. sulph., gms. per kilo.	Chloroform.	Ether.	Pulse. per min.	Respiration per min.
	O_2	CO_2					
24	18.5	40.6	..	..	II	..	..
25	24.0	40.3	..	I	I	..	..
26	16.0	40.1	.010	..	II	..	..
27	20.2	39.9	..	..	II	..	..
28	23.3	39.6	..	..	II	..	..
29	14.3	39.6	.010	..	II	..	..
30	24.6	37.9	..	..	II	..	..
31	15.9	37.4	..	..	II	..	..
32	22.7	37.3	.006	..	II	115	..
33	16.3	37.2	.001	..	II	..	..
34	14.0	36.9	.006	..	II	..	..
35	20.7	36.5	.010	..	II	..	..
36	20.3	36.4	.010	..	II	..	..
37	18.4	36.4	..	..	II	..	..
38	17.0	36.1	..	I	I	150	..
39	15.3	35.1	..	..	II	..	..
40	19.9	33.7	..	I	I	..	..
41	18.4	33.6	..	..	II	..	..
42	24.1	32.7	..	..	II	..	..
43	22.1	31.2	..	..	II	..	..
44	19.9	26.7	..	..	II	..	..
45	20.8	23.1	..	..	II	205	40

I, signifies light anæsthesia, II, moderate, and III, deep.

of the analyses show a CO_2 content which is practically normal. As all of the animals were in a condition of adequate surgical anæsthesia, these cases demonstrate that it is quite possible to avoid both acapnia and hypercapnia, and the dangers which they involve.

It is very easy in a dog under anæsthesia without morphin to raise the threshold above the normal and then to lower it far below. In some dogs this variation can be induced several times within a half hour. Even a few repetitions always result in fatal apnœa. In Fig. 5 are reproduced the pressure-pulse and respiration curves obtained from a dog in which the threshold was thus elevated and depressed. In Table II are shown the data obtainable from the curves and the arterial blood gases at the corresponding times. At first the animal was under adequate but not profound chloroform anæsthesia, which had been induced without excitement. The respiration, pulse, arterial pressure, and arterial CO_2 content were all approximately normal. During the next five minutes the quantity of chloroform was increased until the rate of respiration and pulse were slower by a third, arterial pressure was lowered, and the CO_2 content of the blood was increased by a quarter. Then the administration of chloroform was stopped. After an interval of five minutes, light ether anæsthesia was begun. Ten minutes later the third set of observations were taken. In the curves and table are shown a great acceleration of respiration and pulse, elevation of arterial pressure, and diminution of the CO_2 content of the blood, — in fact, all the typical symptoms of ether-excitement. The eyes were open, and the pupils widely dilated. The animal was entirely unconscious, quiescent except for the respiratory movements, and sufficiently anæsthetized to be irresponsive to all but the most intense afferent irritations.

TABLE II.

EXPERIMENT OF MARCH 15, 1907. SEE FIG. 5.

Time.	Arterial gases.		Respirations per minute.	Heart-rate per minute.	Arterial pressure.	Notes.
	O_2	CO_2			mm. of Hg	
3.10	25.8	41.7	21	70	120	Chloroform anæsthesia.
3.15	14.5	51.1	14	50	100	Deeply chloroformed.
3.30	16.9	34.8	45	130	140	Ether anæsthesia.

Evidently a subject which had been kept for any considerable period in the condition of this dog at 3.30 would develop acapnia. Thereafter, it would be liable to pass into apnœa at any time that deeper ether anæsthesia was induced. Respiratory stand-still would be certain to result from chloroform anæsthesia no deeper than that which was borne with impunity at 3.10. Death would probably follow the administration of much less chloroform than was given at 3.15. If instead of being more deeply anæsthetized the acapnic subject were allowed to come out of anæsthesia, it would necessarily pass through a period of subnormal breathing and cyanosis.

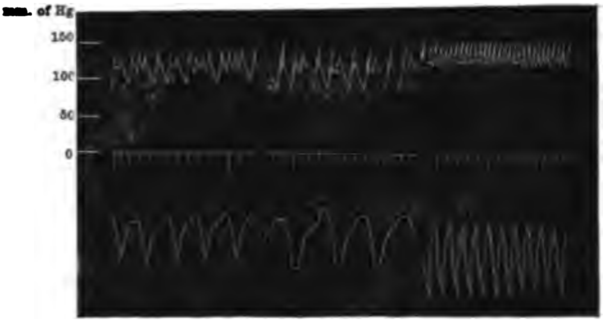

Figure 5. — The original size. Experiment of March 15, 1907. Dog of 8.0 kilos. No morphin. Pressure pulse recorded by a Hürthle manometer connected with the carotid. Time in seconds. Respiration recorded by a spirometer affording a quantitative record of the relative amplitude of breathing in the three periods of observation. Samples of blood for analysis were drawn from the femoral artery, and their gases are reported in Table II. The animal had been anæsthetized quietly with chloroform. At the time of the first record, at the left, it was under full surgical anæsthesia. Five minutes later, when the second record was taken, it was much more deeply chloroformed. The third record, taken fifteen minutes later, shows the conditions occurring after ether had been substituted for chloroform. The degree of anæsthesia, as judged by the corneal reflex and the irresponsiveness of respiration to sensory stimulation, was nearly the same in the third period as in the first. With equal elevations of the afferent threshold the CO_2 threshold was at first normal and later subnormal.

Further experimental evidence in support of these inferences is contained in Sections VI and VII.

VI. Chloroform Apnœa.

Four dogs received 0.01 gm. morphin per kilo body weight. Half an hour later deep chloroform anæsthesia was induced as quickly as possible. There was no hyperpnoea. All four of the animals stopped breathing, because of the sudden elevation of the threshold. No artificial respiration or stimulation was given. We merely kept a finger upon the pulse, in order that aid might be afforded if the heart failed. This precaution, however, proved unnecessary, as care had been used not to administer the chloroform vapor in dangerous concentration. In all four cases spontaneous breathing returned after intervals varying from one and five tenths to four and five tenths minutes. The return of respiration, induced by the accumulation of CO_2 up to the supernormal threshold, was as gentle as that observable in a normal man after apnoea vera induced by voluntary forced breathing. Fatal chloroform apnoea would have resulted from a rise of the threshold requiring more than seven minutes' accumulation of CO_2 and acidosis substances to surmount.

These dogs were later arranged for graphic records of respiration and arterial pressure. Two of them were subjected for twenty minutes to excessive artificial respiration with the double pump described in the fourth paper of this series.[13] With the last few strokes of the pump sufficient chloroform vapor was administered to produce deep, but not excessive, anaesthesia. When the pump was disconnected from the trachea, the dogs lay absolutely quiescent in apnoea, but with normal arterial pressure and heart action of undiminished force for four minutes. Then the heart ceased beating for a few seconds, but resumed at a slower rate. Thereafter the amplitude of the pulse diminished gradually until, after a total of eight minutes of apnoea, the heart ceased beating because of oxygen starvation.

The other two dogs were treated in the manner described in the fifth paper of this series.[14] The sciatic nerve was stimulated electrically for twenty minutes. During this period the animals maintained vigorous pain hyperpnoea. They were maintained in entire unconsciousness in the lower level of the second stage of anaesthesia by continual but moderate administration of ether. At the end of the stimulation enough chloroform was administered to induce the third stage. Both animals passed into apnoea, although the depth of anaesthesia as gauged by the corneal reflex was less than that in which they had been held easily and steadily before the hyperpnoea. The heart

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[illegible]

[illegible]

rate and arterial pressure continued normal during the first four minutes of apnœa. Then both failed but recovered temporarily, only to fail finally in the eighth minute of apnœa, when death occurred.

The graphic records obtained in these two pairs of experiments were similar in all essential features to the curves reproduced in Fig. 6. From the analytical data and other observations of the two preceding papers of this series it is quite certain that: — Before hyperpnœa the arterial blood of these animals contained 40 or more volumes per cent of CO_2. After hyperpnœa, whether with the pump or from afferent stimulation, it contained only about half as much. If air containing small amounts of CO_2 had been introduced into the lungs during the early part of apnœa, the animals would immediately have recommenced breathing. If a jet of oxygen had been blown into the bronchi, asphyxia would have been prevented; and after a prolonged apnœa (twelve minutes or more) spontaneous breathing would have returned. Pharmacologically, these four deaths may be termed chloroform apnœa. Physiologically they were essentially fatal apnœa vera.

VII. Ether-Hyperpnœa.

Four dogs were etherized without previous administration of morphin. After the necessary operations and attachments of recording instruments had been made, the depth of anæsthesia was diminished until respiratory excitement developed. The animals were at this time, as at all times in these experiments, entirely unconscious, and motionless except for the vigorous breathing. After fifteen or twenty minutes of this ether-hyperpnœa deeper anæsthesia was induced. Three of the animals ceased to breathe for a short period, but recovered spontaneously. All four again became hyperpnœic under light anæsthesia. Indeed, the period of apnœa was followed by a marked and prolonged increase in the excitability of the respiratory centre. The threshold for CO_2 was thus lowered and in turn reduced the CO_2 content of the body, until profound acapnia resulted. At the same time the afferent threshold was so high that the animals were irresponsive to, and their breathing was unaffected by any sensory irritation less intense than vigorous electrical stimulation of the sciatic nerve. Finally, a slight increase in the administration of ether, — but no more than had been borne with impunity at the outset, — was followed by a sudden failure of respiration. For four minutes the heart rate continued rapid and the arterial pressure high. Then they failed for a few seconds but recovered temporarily, only to fail finally in the eighth minute of apnoea.

Thus we find that *ether-hyperpnœa is quite as effective as pain-hyperpnœa or excessive artificial respiration as a means of inducing a subsequent fatal apnœa vera.* The dog which withstood this intentionally "unskillful" anæsthesia for the longest period afforded the analytical data and curves reproduced in Table III and Fig. 6.

TABLE III.

EXPERIMENT OF MAY 18, 1909. DOG UNDER ETHER WITHOUT MORPHIN. (SEE ALSO FIG. 6.)

	Volumes.	Per cent.
Arterial blood gases early in ether excitement 3.45 P.M. . . .	22.1 O_2	31.2 CO_2
After twenty-five minutes of ether excitement 4.20 P.M. . . .	19.8 O_2	19.5 CO_2
After death in ether apnœa 5.08 P.M.	0.0 O_2	39.2 CO_2

VIII. VASO-MOTOR AND HEART FAILURE UNDER CHLOROFORM.

One of us recently administered chloroform to a woman for the lancing of a felon on a finger. The subject had suffered intensely for thirty-six hours, and was in an unstrung condition, pale and slightly cyanotic. Although the vapor was given very cautiously, the operation was scarcely begun before the pulse began to fail in rate, tension, and amplitude. At first respiration was not affected. Then it failed also. Death was barely avoided by artificial respiration and massage of the heart.

Such cases are interesting theoretically because laboratory experimentation has generally failed to reproduce them. Lauder Brunton[29] has practically denied that they occur. He held that the investigations of the Bengal Commission demonstrate that "in 99,999 cases out of 100,000 chloroform causes the respiration to fail before it affects the heart." More recently pharmacologists have been led especially

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[29] Brunton, L. T.: Lectures on the action of medicines, 1897, p. 220.

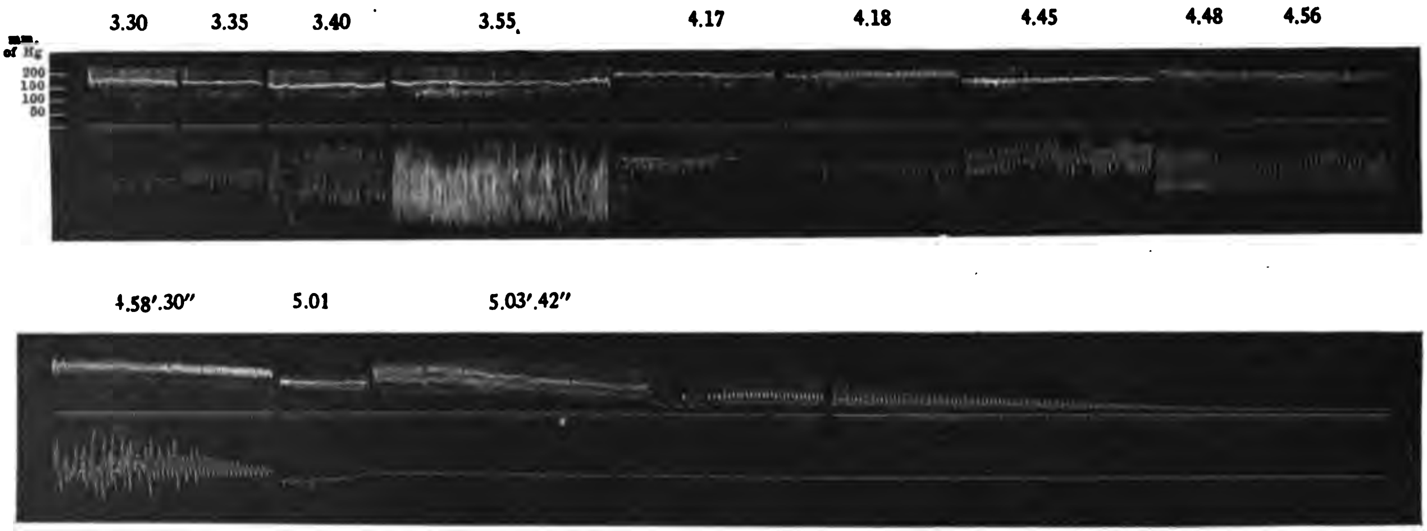

FIGURE 6. — About one fourth the original size. Experiment of May 18, 1909.[1] Record of prolonged ether-hyperpnœa ending with death in apnœa. No morphin. Arterial pressure recorded[*] by a Hürthle manometer connected with the carotid. Time in seconds. Respiration recorded by means of the apparatus described in This journal, 1910, xxv, p. 331. Ether administered after the first anæsthetization by means of the funnel described in This journal, 1910, xxv, p. 390. As the record shows, the dog was continually in hyperpnœa for an hour and twenty minutes, except for an apnœa of twenty-five seconds (at 4.18). Yet the animal was so far anæsthetized that electrical stimulation of the sciatic (4.45 to 4.48) caused only a slight increase in the hyperpnœa. Finally (at 4.58) the respiration became irregular, and then diminished quickly into apnœa. Seven minutes later the heart failed.

by the work of Embley[21]) to admit[22] the correctness of the numerous and well-attested clinical observations upon this topic. The reason for the previous disagreement appears to us to lie in the fact that experimental procedures have not accurately reproduced the clinical conditions under which such cases occur. Doubtless respiration always fails before the heart in normal subjects. This is certainly true of dogs. But the effects of chloroform upon subjects whose immediate past has included sickness and suffering are an altogether different matter. Such subjects are as rare in the laboratory as they are common in the hospital.

Chance has brought under our observation some experimental conditions closely similar to those through which the woman above mentioned had passed. Dogs after being subjected to these conditions are affected by chloroform in the same manner as was she. A very small amount of chloroform caused sudden death in all of the five animals thus treated. *In two cases the circulation failed before the respiration. In the other three the heart action and breathing ceased simultaneously.*

These animals had been used in the experiments imitating the effects of "pain" described in the two preceding papers of this series (q. v.). Two of them had been subjected to excessive artificial respiration for twenty minutes; the other three had been forced into natural hyperpnœa for the same period by stimulation of the sciatic nerve. Thereafter they had lain in apnœa for several minutes, and had then passed through stages of Cheyne-Stokes breathing and shallow respiration. At this time their condition would not have appeared to a casual bystander as notably abnormal, yet a few drops of chloroform induced immediate failure of the circulation, — as illustrated in Figs. 7 and 8.

It is not probable that acapnia was the direct cause of this hypersusceptibility. Comparing these experiments with those described in Section VI, we are led to believe that the essential elements were the prolonged partial anoxhæmia, tissue asphyxia, and intoxication with acidosis substances during the period of recovery from acapnia after the termination of hyperpnœa.

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[21] EMBLEY: British medical journal, 1902, April 5, 12, and 19.

[22] Compare the second edition (1901), pp. 165–167, and the fourth edition (1906), pp. 168–174, of CUSHNY's Textbook of Pharmacology.

IX. Hypercapnia as a possible Factor in Post-Anæsthetic Necrosis.

In normal life the respiratory centre offers a vigorous resistance to the accumulation of an excess of CO_2 in the blood. Haldane and Priestley found that the addition of so little as 0.2 of one per cent of CO_2 to the air inspired induces an increase of 100 per cent in the rate of pulmonary ventilation in a man at rest. Teleologically this fact suggests that any considerable degree of hypercapnia is harmful.

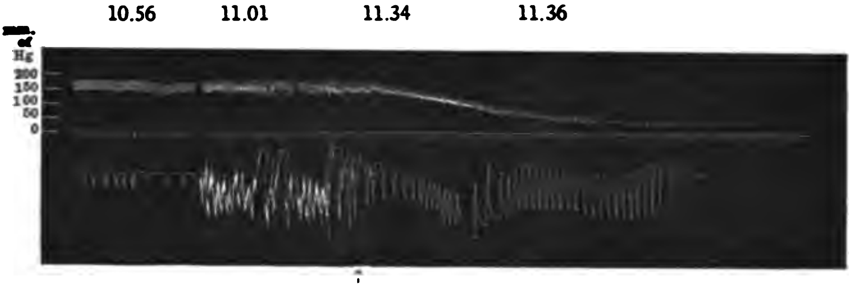

FIGURE 7. — About one third the original size. Experiment of May 29, 1909. Methods of recording and of anæsthetizing were the same as in Fig. 6. No morphin. Under ether the dog had been subjected to stimulation of the sciatic nerve from 11.02 to 11.22. This caused vigorous hyperpnœa and was followed by apnœa for one minute, irregular breathing for two minutes, and then by acapnial shivering. The graphic records of these periods were reproduced in a preceding paper (This journal, 1910, xxv, p. 389). At the point indicated by an arrow five drops of chloroform were placed on the large funnel connected with the trachea. As the record shows, the circulation failed, while respiration continued regularly until the heart stopped.

It has been shown by Bellazzi [23] and by Laqueur [24] that autolysis is greatly accelerated by CO_2. Autolysis and necrosis are now regarded as merely different phases of the same general cytolytic process.[25] The first four experiments cited in Table I show that in dogs in profound morphin-chloroform anæsthesia, or even under chloroform alone, the arterial blood may contain a quarter to a half more than the normal quantity of CO_2. It is significant that post-anæsthetic necrosis is seldom a consequence of ether, since this "respiratory stimulant"

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[23] BELLAZZI, L.: Zeitschrift für physiologische Chemie, 1909, lvii, p. 389.

[24] LAQUEUR, E.: Schriften der physik.-ökonom. Gesellschaft zu Königsberg i. Pr. 1909, Jahrg. L, i, 3.

[25] See WELLS, H. G.: Chemical Pathology, 1907, pp. 88–103, 307–331, and 457 (full bibliography).

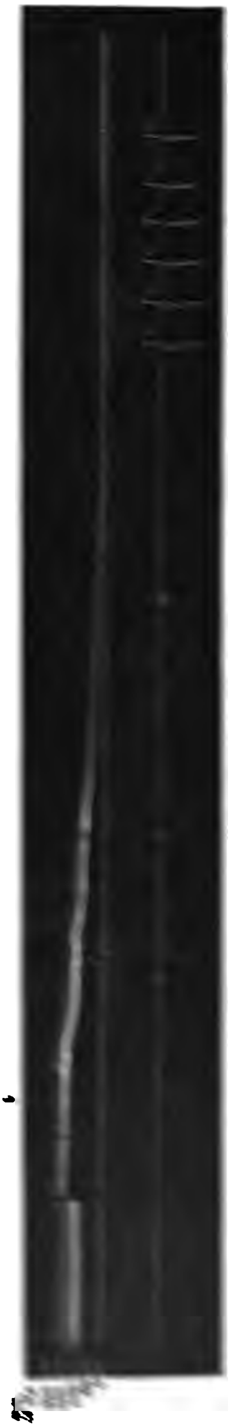

Fig. 7. [illegible] Experiment of June 11, 1909. Methods of recording and of anesthetizing were the same as in Fig. 6. [illegible] of morphin sulphate per kilo, and had then been kept under ether. From 10.45 to 11.15 the sciatic nerve was [illegible] nearly continuous hyperpnœa. Thereafter the dog lay in apnœa for five minutes, then drew a single breath [illegible] apnœa for another six minutes. It recovered through stages of Cheyne-Stokes breathing and shallow respiration. At 1.02 its [illegible] not markedly abnormal, yet the administration of a few drops of chloroform caused both circulation and respiration to fail. A [illegible] as we have repeatedly demonstrated, would take this quantity of chloroform with impunity. Two minutes after the heart had stopped, [illegible] pump inspirations. For the blood gas analyses of this experiment, see This Journal, 1910, xxv, p. 404.

prevents hypercapnia. On the other hand, tissue degenerations sometimes occur not only after chloroform, but after morphin narcosis and after nitrous oxide. The effects of chloroform have usually been regarded as due to the direct toxic action of the drug upon living cells. Neither morphin nor nitrous oxide are protoplasmic poisons, yet cases are on record in which they induced results similar to those of chloroform. These considerations support the view that *intense and prolonged hypercapnia during anesthesia may be the factor which induces, or aids in producing, post-anesthetic necrosis.*

X. CONCLUSIONS.

I. Anesthetics tend to prevent shock because they diminish pain-hyperpnœa and thus obviate the development of acapnia.

II. Respiratory excitement during the initial stages of anesthesia diminishes the CO_2 content of the blood and thus tends to induce a subsequent failure of respiration. Similarly ether, unless neutralized by morphin, often causes in dogs a persistent acapnia and a consequent true apnœa.

III. Morphin raises the threshold for CO_2 more than it does the afferent threshold of the respiratory centre. Chloroform elevates the latter threshold more than the former. Ether in quantities short of profound anæsthesia exerts a "respiratory stimulant" influence which lowers the threshold for CO_2 and thus tends to induce acapnia.

IV. Apnœa in anæsthesia depends, in the same manner as in normal life, upon the relation of the level of the threshold of the respiratory centre for CO_2 to the quantity of CO_2 in the blood and tissues. Whenever the former is above the latter, spontaneous breathing ceases.

V. Under anæsthesia the threshold for CO_2 may be elevated 50 per cent above normal, or depressed 50 per cent below normal. Such a depression of the threshold causes vigorous hyperpnœa. If long continued, it results in intense acapnia.

VI. Chloroform apnœa may be regarded as merely a form of apnœa vera.

VII. Experiments show that ether-hyperpnœa is quite as effective as pain-hyperpnœa as a means of inducing a subsequent fatal apnœa vera.

VIII. In normal subjects under chloroform respiration always fails before the heart. Subjects which have passed through a period of sickness and suffering, or their experimental equivalents, are hyper-susceptible to the toxic influences of chloroform. In such cases the circulation fails first, or simultaneously with respiration.

IX. Hypercapnia during anæsthesia may be the factor which determines the development of chloroform necrosis.

X. Skillful anæsthesia consists in maintaining the threshold of the respiratory centre for CO_2 at a nearly normal level, and in avoiding the development either of acapnia or of hypercapnia.

NOTE. — Of great importance for the acapnia theory are the recent papers of KROGH showing that the gaseous exchanges in the lungs are due simply to diffusion, and are not secretory. If BOHR's theory of pulmonary gaseous secretion were correct, the basis of our discussion would be invalidated. It is only fair to add, also, that we agree with KROGH in upholding the truth and the importance of both of the other ideas on this subject supported by BOHR. — (1) That the heart is incapable of imparting to the blood stream any such velocity as ZUNTZ and HAGEMANN and their followers have assumed. And (2) that at times a very considerable metabolism (disappearance of oxygen and production of CO_2 incident

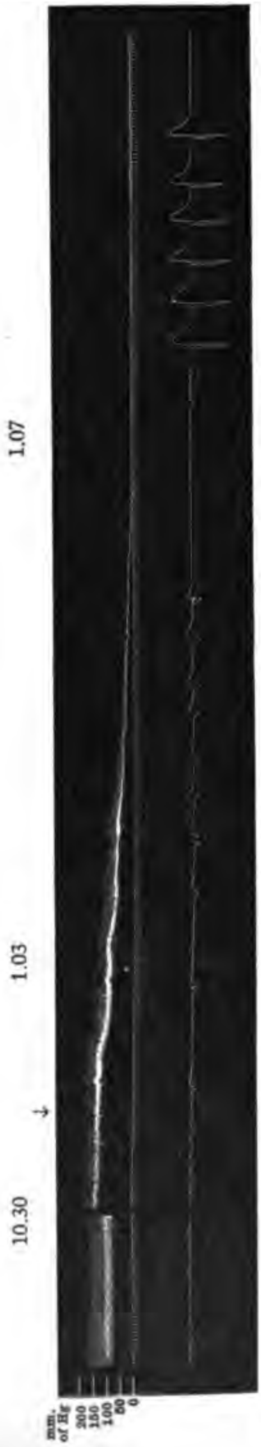

FIGURE 8. — About one third the original size. Experiment of June 23, 1909. Methods of recording and of anæsthetizing were the same as in Fig. 6. The dog had received 0.015 gm. of morphin sulphate per kilo, and had then been kept under ether. From 10.45 to 11.15 the sciatic nerve was stimulated electrically so as to induce nearly continuous hyperpnœa. Thereafter the dog lay in apnœa for five minutes, then drew a single breath and relapsed into apnœa for another six minutes. It recovered through stages of Cheyne-Stokes breathing and shallow respiration. At 1.02 its condition was not noticeably abnormal, yet the administration of seven drops of chloroform caused both circulation and respiration to fail. A normal dog, as we have repeatedly demonstrated, would take this quantity of chloroform with impunity. Two minutes after the heart had stopped, the animal drew six gasping inspirations. For the blood gas analyses of this experiment, see This journal, 1910, xxv, p. 394.

prevents hypercapnia. On the other hand, tissue degenerations sometimes occur not only after chloroform, but after morphin narcosis, and after nitrous oxide. The effects of chloroform have usually been regarded as due to the direct toxic action of the drug upon living cells. Neither morphin nor nitrous oxide are protoplasmic poisons, yet cases are on record in which they induced results similar to those of chloroform. These considerations support the view that *intense an prolonged hypercapnia durin anæsthesia may be the factor whi induces, or aids in producin post-anæsthetic necrosis.*

X. CONCLUSIONS.

I. Anæsthetics tend to p vent shock because they di ish pain-hyperpnœa, and obviate the developmen acapnia.

II. Respiratory excit during the initial stag anæsthesia diminishes th content of the blood, an tends to induce failure of respi ether, unl morphin hyper seq [illegible] elevates the latter threshold more than the [illegible] quantities short of profound anesthesia [illegible] [illegible]" influence which lowers the threshold [illegible] to induce acapnia.

V. Apnoea in anesthesia [illegible] [illegible] upon the [illegible] [illegible] [illegible] Whenever the [illegible] [illegible]

[illegible] above normal [illegible] suppression of the [illegible] [illegible] it results in [illegible]

VI. Chloroform [illegible]

VII. Experiments [illegible]

VIII. In normal [illegible]

to a combustion of acidosis substances) occurs in the lungs. *Cf.* KROGH: Skandinavisches Archiv für Physiologie, 1910, xxiii, pp. 258 and 275. BOHR: Same journal, 1909, xxii, p. 221. See also HENDERSON, Y.: This journal, 1909, xxxii, p. 360, and 1910, xxv, pp. 328 and 392; also Proceedings American Physiological Society, Same volume, p. xii.

ADRENALECTOMY AND GLYCOSURIA.

By HUGH McGUIGAN.

[From the Laboratory of Pharmacology, Washington University, St. Louis.]

IT is well known that adrenalin, when injected intravenously, hypodermically, or intraperitoneally, causes glycosuria. Adrenalemia, however produced, would doubtless act in the same way. In keeping with this statement, Waterman and Smit[1] found that in normal animals piqure puncture caused both glycosuria and an increase in the adrenalin content of the blood. If adrenalemia causes glycosuria, to remove the excess of adrenalin, or better to cause an adrenalin deficiency, might throw some light on the mechanism of glycosuria. A study of the effects of reducing the adrenalin content of the blood was made by A. Mayer,[2] who found that after adrenalectomy piqure puncture did not cause glycosuria. Nishi,[3] in confirming this result, adds that the adrenals are also necessary for the production of glycosuria by diuretin. If the above work be correct, it is evident that there is a close relation between the secretion of the adrenals and glycosuria.

There seemed to me to be several reasons why the above statements should not be accepted without further investigation. 1. The work was done on rabbits — animals especially susceptible to shock — and it is difficult to separate the phenomena of shock and adrenal deficiency. 2. From the work of Strehl and Weiss[4] we should expect that the removal of the adrenals would render the secretion of the urine impossible and therefore create conditions adverse to the study of glycosuria. 3. A possible cutting of the splanchnic nerves, and a confusion of the effects of this with the effects of removing of the adrenals. 4. My own failure to find an unquestionable change in the contractions of the uterus in the serum taken before and after adrenalectomy.

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[1] WATERMAN and SMIT: Archiv für die gesammte Physiologie, 1908, cxxiv, p. 198.

[2] MAYER, A.: Comptes rendus de la Société de Biologie, 1906, pp. 1123–1124.

[3] NISHI, M.: Archiv für experimentelle Pathologie und Pharmacologie, 1909, lxi, p. 401.

[4] STREHL and WEISS: Archiv für die gesammte Physiologie, 1901, lxxxvi, p. 107

The object of the present paper is to report work which shows that general conclusions regarding the production of glycosuria cannot be drawn from work done on rabbits alone; that various species of animals react differently; that the removal of the adrenals does not necessarily influence the mechanism of salt glycosuria.

METHODS.

Ether was used as the anæsthetic for all animals; in a few rabbits 2 gm. to the kilo of urethane was also used. The abdomen was opened in the middle line by an incision extending from the ensiform process to the pubes. The intestines were enveloped in towels wrung out of warm salt solution (0.9 per cent). When advantageous, a transverse incision over the level of the kidneys was also made. The intestines were drawn to the side and when necessary out of the abdomen. The adrenals were dissected out, the vessels tied, and the glands removed. The operation took from five to fifteen minutes. In most cases the stumps were cauterized with a hot iron. The abdomen was closed, sutured, and the animals kept warm during the experiment. The urine was obtained by pressure on the bladder. The tests for sugar were made by Fehling's solution and by fermentation. The blood pressure was taken from the carotid in the usual way.

EXPERIMENTS WITH RABBITS.

So far as the results with rabbits are concerned, they are in harmony with those of Mayer and Nishi. In a few cases I found sugar after adrenalectomy, but in each case the experiment was open to question. The general statement that after adrenalectomy no sugar appears in the urine of rabbits, seems to be correct.

Experiment. November 18, 1909. — Rabbit, weight 1870 gms. Blood pressure not taken.

11.30. Given 2 gm. urethane per os.

11.45–12.00. Adrenals removed, except one-third of the right gland and cannula placed in the superior mesenteric vein and the abdomen closed.

12.25–35. 50 c.c. 0.9 per cent NaCL ran into vein. Bladder emptied, no sugar.

1.12–1.25. 125 c.c. *m*/2 Na_2SO_4 was run into the vein.

1.25. 50 c.c. of urine was collected and contained a large amount of sugar.

Post mortem examination showed that one-third of the right adrenal had not been removed.

The result of this experiment can be taken to show that this strength and amount of solution will cause diuresis and glycosuria in the rabbit. The presence of the small amount of gland is sufficient to render possible the production of glycosuria. The part remaining was tied off, and it is questionable whether the sugar was due to its presence.

No ember 24, 1909. — Rabbit, weight 2300 gm.

12.00. Given 2 gm. urethane per os.

1.10–1.25. Given ether and the adrenals removed. Urine contained no sugar.

1.50–2.55. 225 c.c. *m*/2 Na_2SO_4 had run into the portal vein.

3.05. Animal dead. 7 c.c. urine contained no sugar.

Post mortem. No adrenal tissue remained. The intestines were full of fluid.

In all eighteen rabbits were used, and from 200 to 270 c.c. of salt solution injected (*m*/2 Na_2SO_4). The salt injection was usually made into the jugular vein. No other case than the one quoted above gave a positive salt glycosuria. In many cases ether alone was used, no urethane being given.

The following shows the effect of adrenalectomy on the blood pressure of rabbits.

January 8, 1910. — Rabbit, male, 1800 gm.

10.15. Ether.

10.23. Blood pressure in carotid 100 mm. Hg.

10.53. Abdomen opened, intestines protected with warm towels wrung out of 0.9 per cent NaCl. Dissected around the adrenals in the same manner as for their removal. Abdomen closed.

10.55. Blood pressure 50 mm. Hg.

11.02. Blood pressure 54 mm. Hg.
11.12–11.15. Adrenals removed.
11.18. Blood pressure 30 mm. Hg.
11.22. One-half c.c. 0.1 per cent strychnine sulphate subcutaneously.
11.40. Blood pressure 58 mm. Hg.

The pressure here was probably very little influenced by the strychnine, as I obtained a similar rise in each case when no drug was given. I have never seen the rise attain more than 60 mm. without the injection of salt solution.

Does the permeability of the kidneys of normal and adrenalectomized rabbits differ?

The following experiments answer this question:

November 11, 1909. — Rabbit, 2300 gm. Urethane and ether anæsthesia.
11.25. Adrenals removed and abdomen closed.
12.15. 2 gm. of dextrose in 100 c.c. 0.9 per cent NaCl ran into jugular vein.
12.30. 15 c.c. urine squeezed from the bladder contains a large amount of sugar.
2.20. Bladder again emptied, 5 c.c. urine, large amount of sugar.
6.35. Animal still alive, but no urine in the bladder.
9.00 A. M. next day. Animal found dead, bladder contained no urine.

The experiment was repeated using 0.5 gm. of sugar in a rabbit weighing 1600 gm. In this case no sugar appeared in the urine.

Calculating the amount of blood in the rabbit at 5 per cent of the body weight, there was at least 1 per cent sugar in the blood of the first rabbit and 0.25 per cent in that of the second. The result agrees with that found by Blumenthal[5] and Comessati[6] for normal animals. They found that it was possible to inject over one gram of dextrose per kilo body weight before sugar appeared in the urine of rabbits. The results obtained by injection into the general circulation, however, must not be confounded with the results of injections made directly into the renal artery.[7] In this later case the smallest amount will be eliminated promptly by the kidneys.

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[5] **Blumenthal**: Beiträge zur chemischen Physiologie und Pathologie, 1905, vi, p. 329.

[6] **Comessati**: *Ibid.*, 1907, ix, p. 67.

Action of phloridzin after adrenalectomy in rabbit.— I. A normal animal, weight 1500 gm., was given one half gram phloridzin subcutaneously. A large amount of sugar appeared in the urine in a few hours.

II. Weight of animal 1150 gm. Ether anæsthesia.
9.00–9.25. Adrenals removed and abdomen closed.
9.30. 0.5 gm. phloridzin was given subcutaneously.
10.00. 10 minims of 0.1 per cent strychnine sulphate subcutaneously.
1.00. Animal in good condition. No urine.
1.45. 0.25 gm. phloridzin subcutaneously.
3.30. Animal dead. Bladder contains 5 c.c. urine. Large reduction and fermentation.
Post mortem showed that all adrenal tissue had been removed.

Two other animals treated similarly, but without the strychnine, died in three hours without having secreted urine. The short duration of life may be partially caused by a greater toxicity of phloridzin after adrenalectomy.

The positive result after phloridzin, in contrast to the negative result of Na_2SO_4, may be explained on the accepted theory that the mechanism of the glycosuria in the two is different. A different theory, however, need not be invoked.

It is generally accepted that the sugar in the blood exists in combination. A small amount may be free. Salts probably alter the ratio of the free to the combined sugar, and when the free sugar reaches a certain amount,— which is constant, — glycosuria results. The organs of the body have a certain combining power for free sugar. This combining power may also be lessened by salts that cause glycosuria. Phloridzin may affect the ratio of the free to the combined sugar more than other drug, and for that reason glycosuria could result without an increase in the total quantity of the blood being changed, and without accepting the questionable theory that the kidneys manufacture the sugar in phloridzin glycosuria. This, however, is pure theory.

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[7] Underhill and Kleiner: Journal of biological chemistry, 1908, iv, p. 395.

EXPERIMENTS WITH DOGS.

Influence of the removal of the adrenals.

January 19, 1910. — Dog, 7200 gm. Ether anæsthesia.

1.30–2.30. Cannulæ in the trachea, jugular, and carotid and the adrenals removed. Abdominal aorta clamped below the kidneys, and abdomen closed.

2.40. Bladder emptied, urine contained no sugar.

2.44. Commenced to inject $m/2$ Na_2SO_4 into jugular . . . . B. P. 40 mm. Hg.
" rising.

2.48. 40 c.c. more salt solution in vein . . . . . " 82 mm.

2.53. 40 c.c. more in . . . . " 126 mm.

2.58. 40 c.c. more in . . . . " 140 mm.

3.03. 40 c.c. more in . . . . " 130 mm.

3.08. 40 c.c. more in . . . . " 130 mm.

3.13. 40 c.c. more in . . . . " 130 mm.

3.18. 16 c.c. urine, contains no sugar.

3.19. 40 c.c. more salt solution in . . . . " 110 mm.

3.26. 40 c.c. more salt solution in . . . . " 104 mm.

3.32. 16 c.c. urine contains trace of sugar.

3.36. 40 c.c. more salt solution in . . . . " 120 mm.

3.48. 20 c.c. urine, gives considerable reduction and ferments.

3.55. Pressure almost to zero and animal dying.

Post mortem showed that all the adrenal tissue had been removed.

Similar results were obtained with augurin.

In all five dogs were used. Two gave positive results, and three negative.

It can safely be said that removal of the adrenals in dogs renders glycosuria harder to elicit, but it can be produced.

EXPERIMENTS WITH CATS.

December 16, 1909. Animal not weighed. Ether anæsthesia.

11.50. Cannulæ in the femoral vein and carotid artery. Bladder emptied. Urine contains no sugar.

11.55. Adrenals removed. After removal . . B. P. 110 mm. Hg.

12.30–12.40. 120 c.c. $m/8$ Na_2SO_4 injected. . . " 135 mm.

12.45. Urine commences to run from the bladder. Contains a large amount of sugar . . . . . " 76 mm. Hg.

12.49. 40 c.c. more salt solution ran into the vein.
1.00. Bladder full of urine which contains a large amount of sugar . . . . " 76 mm. Hg.
Animal killed. Post mortem showed that the adrenals had been completely removed.

The experiment was repeated with like results. Salt glycosuria is rapidly and easily produced in the cat after the removal of the adrenals. A difference in the susceptibility of the cat as compared with the dog or rabbit is quite apparent. $m/2$ Na_2SO_4 is very toxic for the cat when injected into the vein.

DISCUSSION OF RESULTS.

With rabbits, removal of the adrenals renders salt glycosuria impossible. That this is not a true deficiency phenomenon, but a result of some secondary factor, is rendered probable from results obtained with dogs and cats. Each of these animals gives positive results. In the cat, expecially, salt glycosuria can be rapidly and easily produced. The cause of the difference between the cat and the rabbit seems to be due to different degrees of shock. As measured by the blood pressure, shock is greater in the rabbit than in the cat; this element alone may be sufficient to explain the different response to salt action. The difference might be due to the presence of accessory glands in the cat and their absence in the rabbit, although I have been unable to find evidence in favor of this possibility. The short duration of life would speak against their presence, and even if present, it is questionable whether on such short notice they could functionate to such a degree as to cause the great difference in results.

There is a decided difference in the susceptibility of these animals to sodium sulphate; rabbits and dogs being remarkably tolerant, while cats are easily killed by an action on the heart. A $m/2$ solution can be injected into the rabbit or dog with impunity but will kill a cat instantly. The difference here seems to be as great as the difference in the susceptibility of dogs and rabbits to chloroform.

The fact that sugar, when injected intravenously, passes through the kidneys as in a normal animal, shows that the kidneys are little, if any, changed. Phloridzin also causes glycosuria as in a normal animal. The usual statement that phloridzin causes glycosuria by acting on the kidneys may be considered a sufficient explanation for its action here. There is, however, no sufficient reason for assuming one mechanism for phloridzin glycosuria and another for the caffein compounds or salt solutions. The reason why phloridzin causes glycosuria when sodium sulphate solution or a caffein compound does not, probably is due to quantitative differences caused in the ratio of the free to the combined sugar in the blood; phloridzin may cause a larger amount of the free sugar. This, however, must remain an hypothesis until a quantitative method is devised to determine the condition of the sugar in the blood.

When the duration of life is so short after removal of the adrenals, it is difficult to assign a true value to negative results. Rabbits live on an average of eight to fourteen hours after the operation, and during this time they never fully recover from the immediate results of the operation. It is quite impossible then to differentiate the effect of shock and adrenalin deficiency. With dogs and cats, however, the phenomenon of shock — at least so far as the blood pressure is a measure — is not so great, and positive results can be obtained with these animals.

SUMMARY.

1. Removal of the adrenals in rabbits renders the production of salt glycosuria impossible, while phloridzin glycosuria occurs readily.
2. Removal of the adrenals in dogs makes the production of salt glycosuria more difficult, but it can be produced.
3. Removal of the adrenals in cats does not seem to modify the production of salt glycosuria.

NOTES ON THE ANALYSIS OF EDESTIN AND ZEIN.

By THOMAS B. OSBORNE and L. M. LIDDLE.

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OWING to a fire which recently destroyed our laboratory, some of the work in progress at the time could not be completed, but as data had been obtained which give some information concerning losses that occur in estimating the products of hydrolysis of proteins, and also give additional data respecting the amount of some of the decomposition products of edestin and zein, we have thought it worth while to publish them, although the analyses are far from complete.

HYDROLYSIS OF EDESTIN.

Two portions of 100 gm. each, and one of 300 gm. of air-dry edestin were separately hydrolyzed by heating on a water bath with 1000 c.c. of hydrochloric acid, sp. gr. 1.1, and then by boiling in an oil bath for twenty-four hours. The total quantity of edestin equalled 444 gm. ash and moisture free.

Each solution was separately concentrated to about one-half its volume and then saturated with hydrochloric acid gas. After standing on ice for six days the glutaminic acid hydrochloride was filtered out, dissolved in water, the solutions decolorized with bone coal and concentrated to crystallization. The quantity of pure hydrochloride obtained from these solutions was equal to 12.89, 12.36, and 36.45 gm. of free glutaminic acid or to 14.50, 13.91, and 13.67 per cent respectively.

These figures agree well with the earlier determination by Osborne and Gilbert[2] who found by direct separation 14.0 per cent and also

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[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] OSBORNE and GILBERT: This journal, 1906, xv, pp. 333-356.

with a later result of 14.5 per cent by Abderhalden (communicated by letter). These results are however all too low, for after returnin the mother liquors from the re-crystallization to the main hydrolysis solution we subsequently isolated from the esters a quantity of glutaminic acid equal to 4.84 per cent, making the total yield 18.74 per cent of the combined amount of edestin which was hydrolyzed.

In this connection attention should be called to the fact that the 6.3 per cent of glutaminic acid which was reported by Abderhalden,[3] and since extensively quoted in the literature, was obtained by the ester method several years ago, and at a time when it was not known that much of the glutaminic acid ester is retained in the distillation residue.

The main hydrolysis solution, together with the mother liquors from the glutaminic acid, was largely freed from water by twice evaporating at a low pressure with much absolute alcohol, and esterified by adding 35 gm. of zinc chloride, and passing the vapors of 1500 c.c. of absolute alcohol through the solution at 105° during six and one-half hours. The esters were liberated according to Fischer's method and dried over sodium sulphate.

The residue from which the esters had been extracted with ether was freed from inorganic salts in the usual way, and the amino-acids again esterified and shaken out with ether as before.

After esterifying for a third time, the united ethereal solutions of the esters were freed from ether by distilling from a water bath at atmospheric pressure, the distillate was acidified with alcoholic hydrochloric acid, and after standing some time, a precipitate was filtered out. The acidified ether filtrate was shaken with water and the aqueous layer used to dissolve the precipitate produced by alcoholic hydrochloric acid. The resulting solution was freed from ammonia by boiling with an excess of baryta, the baryta removed, and the solution evaporated three times with absolute alcohol. The final residue was dissolved in absolute alcohol and the solution saturated with gaseous hydrochloric acid. After seeding with glycocoll ester hydrochloride it yielded 8.53 gm. of this substance.

The mother liquor was then heated on a water bath with an excess of baryta, the barium and chlorine removed in the usual way, the solution decolorized with bone coal and concentrated. By fractional crystallization this solution yielded 2.46 gm. of leucine, 3.67 gm. of alanine, and 3.97 gm. of a mixture which was added to Fraction I, subsequently obtained by distilling the esters.

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[3] ABDERHALDEN: Zeitschrift für physiologische Chemie, 1903, xl, p. 249.

These results show that a not inconsiderable loss of amino-acids, especially of alanine, has occurred in past analyses of proteins, through neglect to recover that part of them carried over with the ether.

That leucine ester is thus easily carried over with the ether was later confirmed in connection with another experiment, in which 30 gm. of leucine and 15 gm. of aspartic acid were esterified and the esters shaken out with ether according to Fischer's method. Although the volume of the ether was only one litre, after distilling it from the esters at atmospheric pressure, we recovered from it 1.76 gm. of leucine. From this result it appears that considerable losses of amino-acids may occur when the ether distilled from the esters amounts to several litres, as is usually the case when 400 to 500 gm. of protein are hydrolyzed.

The total yield of crude esters from which the ether had been distilled was 354 gm. or 80 per cent of the edestin hydrolyzed. In view of the fact that edestin contains at least 18 per cent of basic amino-acids, and that 14 per cent of glutaminic acid had been removed before esterifying, this yield compares favorably with the yield of esters obtained from zein by Phelps and Tillotson's method. It is evident from this, that a satisfactory esterification can be obtained by this method, with proteins yielding much basic amino-acids, as well as with those yielding but little.

That a relatively considerable quantity of esters was obtained from the third esterification, namely 82 gm., was due to the long time required to dissolve the hydrochlorides of the esters in the small quantity of cold water used according to Fischer's directions, before liberating the free esters with sodium hydroxide. We have always encountered this difficulty in analyzing proteins yielding much arginine, and there can be no doubt that in such cases hydrolysis of the esters occurs to considerable extent during this process.

The esters were then distilled in vacuo with the following results.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	110°	2.0 mm.	104.82 gm.
	condensed by liquid air	....	43.68 "
II	160°	1.5 mm.	94.81 "
	condensed by liquid air	....	6.89 "
Undistilled residue			84.00 "
Total			334.20 gm.

Fraction I, when saponified as usual, was evaporated to dryness and the residue extracted with absolute alcohol. The amino-acids insoluble in alcohol were destroyed by the fire before their separation was completed.

The alcoholic extract was freed from all substances insoluble in alcohol, by repeatedly evaporating to dryness, and extracting the residue with absolute alcohol as long as anything insoluble in alcohol remained. The proline was then converted into the copper salt, the latter extracted with absolute alcohol, and the alcoholic solution freed from all copper salts insoluble in alcohol, by repeatedly evaporating and extracting the residue with absolute alcohol. By this treatment 22.86 gm. of l-proline copper, dried at 110° were obtained, equal to 18.03 gm. of free l-proline. The copper salts insoluble in alcohol yielded 0.3 gm. of pure r-proline copper equal to 0.21 gm. of free proline. The total quantity of proline thus isolated is equal to 4.10 per cent of the edestin or more than twice as much as the 1.7 per cent reported by Abderhalden.

Fraction II, when shaken out with ether and otherwise treated in the customary way, yielded 16.78 gm. of phenylalanine hydrochloride, equal to 13.75 gm. of the free acid, or 3.09 per cent.

The undistilled residue when worked up for glutaminic acid in the usual way yielded 26.85 gm. of the hydrochloride, equal to 21.51 gm. of the free acid, or 4.85 per cent.

The residue which remained after shaking out the esters for the third time was freed from inorganic salts and examined for oxyproline according to the method used by Emil Fischer in isolating this amino-acid from gelatin.[4] After filtering out some tyrosine which separated from the concentrated solution, from which all regents and basic amino-acids had been carefully separated, the solution was decolorized with animal charcoal and concentrated to a small volume. After long standing over sulphuric acid a crystalline separation occurred, but this showed every indication of a mixture.

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[4] FISCHER: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 2660.

Although much time was spent in endeavoring to separate some definite product, no oxyproline or other amino-acid was obtained in sufficient purity for identification. We observed no difference between the behavior of this solution and that of similar solutions which we have examined from other proteins, and feel satisfied that the small amount of material contained, at the most, only insignificant quantities of oxyproline. We see no reason why we should not have obtained this substance if it were present in the proportion reported by Abderhalden. In view of the meagre evidence which Abderhalden gives as to the identity of the substance isolated by him, we consider that further proof is needed to show the presence of oxyproline among the decomposition products of edestin.

ANALYSIS OF ZEIN.

In esterifying according to Phelps and Tillotson's method, zinc chloride is used as a catalyzer. To learn whether or not equally good results could be obtained without using this reagent, we undertook another analysis of zein, the partial results of which are here given. In making this analysis we also wished to learn whether or not losses might be avoided by using sodium alcoholate to liberate the esters in the manner employed by Abderhalden and his associates, when working with small quantities of material. We also wished to see if a higher yield of alanine and other amino-acids could be obtained by taking precautions to recover all the amino-acids carried over with the alcohol and ether when these solvents were distilled off from the free esters.

A quantity of air-dry zein, equal to 184.6 gm. of moisture, ash and fat-free substance, was hydrolyzed by boiling with 500 c.c. hydrochloric acid for forty-nine hours. After removing 36.88 gm. of glutaminic acid hydrochloride, the filtrate from the first separation of the glutaminic acid hydrochloride and the mother liquors from its re-crystallization were united and evaporated to a syrup. After treating with alcohol 13.33 gm. of ammonium chloride were filtered out. The alcoholic solution was saturated with hydrochloric acid, evaporated under diminished pressure to a syrup, and 8.45 gm. more ammonium chloride filtered out.

The amino-acids in the alcoholic solutions were then esterified by passing through the solution the vapors of 1100 c.c. of absolute alcohol containing 44 c.c. of alcoholic hydrochloric acid. The solution containing the esters was then made up to 500 c.c., chlorine estimated in 5 c.c. of it and the esters liberated by adding a solution of sodium ethylate containing a very little less than the calculated quantity of sodium.

After the sodium chloride had settled during the night, the clear solution was decanted and an equal volume of ether was added to the residue. This was then left on a folded filter over night.

The solution of the esters was evaporated under diminished pressure, the condensed alcohol and ether being collected in a flask, which in turn was connected with a second flask containing hydrochloric acid in order to hold any esters that might be carried over with the vapors which had failed to condense.

The esters, from which the alcohol and ether had been distilled, were dissolved as far as possible in absolute ether. A part which did not dissolve was filtered out and treated with water, alcoholic hydrochloric acid added, and the solution evaporated to a thick syrup, which was taken up in alcoholic hydrochloric acid. The sodium chloride, which had been filtered from the alcoholic solution of the esters, and which still contained much adhering esters, as well as unesterified portions of the amino-acids, was dissolved in hot water, the solution made acid with hydrochloric acid and evaporated to a syrup. The residue was treated with alcohol, the sodium chloride filtered out, and the solution added to the solution similarly obtained from the part of the esters which did not dissolve in the ether.

After evaporating the united solutions to small volume, the dissolved substances were re-esterified by passing the vapors of 700 c.c. of absolute alcohol, containing 50 c.c. of alcoholic hydrochloric acid, through the mixture during five hours.

The solution was then evaporated, under diminished pressure, to a syrup, in order to remove the excess of hydrochloric acid, the residue dissolved in absolute alcohol, the solution made up to a volume of 500 c.c. and chlorine determined in 5 c.c. The esters were then set free by adding 0.5 gm. less than the calculated quantity of sodium, which was dissolved in 500 c.c. of absolute alcohol. After standing over night, the clear solution was drawn off, and the sodium chloride filtered from the remainder, and washed with absolute alcohol. The alcohol was then distilled off under diminished pressure, using the same precautions to recover any esters that were carried over as described for the first esterification. The residue of esters was then extracted with absolute ether, and the filtered ethereal solution united with the corresponding solution of the esters obtained by the first treatment.

In order to determine how much esters were carried off with the alcohol distilled from the esters, the acid distillates were evaporated under diminished pressure, and the residues from each esterification were examined separately as follows:

The residue from the alcohol distilled from the first esters was taken up in water, an excess of baryta added, and the solution evaporated to expel ammonia. After twice repeating the evaporation with water, the barium was removed with sulphuric acid, the chlorine with silver sulphate, and the sulphuric acid with an equivalent quantity of baryta. From this solution 10.52 gm. of amino-acids were recovered, of which 1.86 gm. were leucine, 4.34 gm. alanine, and 4.32 gm. a mixture of leucine, valine and alanine, which were united with a similar fraction obtained from the esters subsequently distilled at low pressure.

The ether solution of the esters was subjected to distillation from a water bath at atmospheric pressure and the ether distillate shaken up with dilute hydrochloric acid and evaporated to dryness.

During the subsequent distillation of the esters at low pressures, liquid air was used throughout the process, to condense any vapors that otherwise escaped condensation. The substance thus condensed was acidified with hydrochloric acid and likewise evaporated to dryness. The residue, 2.17 gm., was united with the residue from the ether, and also with the residue from the acidified alcohol distilled from the products of the second esterification.

The united substances were freed from ammonia and otherwise treated in the same way as the acidified distillate from the first esters. By fractional crystallization of the free amino-acids 2.81 gm. of a mixture were separated which consisted chiefly of leucine and valine (?), 3.99 gm. of alanine and 3.09 gm. of a very soluble fraction. This latter and also the 2.81 gm. of mixed acids were added to a similar fraction obtained by subsequently distilling the esters at low pressure.

We thus find that a total of 22.49 gm. of amino-acids were carried over with the alcohol and ether distilled from the free esters or 12.2 per cent of the zein. These figures show that serious losses may occur if due precautions are not taken to recover the amino-acids which are carried off when the solvents are distilled from the free esters, and that much of this loss falls on alanine.

DISTILLATION OF THE ESTERS.

The esters which remained after distilling off the ether weighed 174 gm. equal to 94.0 per cent of the zein even though 16 per cent of glutaminic acid had been previously removed as hydrochloride. We thus find that the yield of esters was quite as great as the best yields which we had previously obtained when zinc chloride was used as a catalyzer, and that this reagent is not necessary in effecting a satisfactory esterification. The esters were then distilled as rapidly as possible, consistent with good condensation, liquid air being used throughout the entire distillation in order to condense everything that passed over. Only 2.17 gm. of residue was obtained by evaporating the contents of the liquid air tube acidified with hydrochloric acid.

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	95°	1.3 mm.	41.14 gm.
II	105°	0.9 "	38.42 "
III	115°	1.2 "	3.86 "
	Distillation residue	....	75.00 "
	Condensed by liquid air	....	15.06 "
Total			173.48 gm.

Fraction I was saponified in the usual way, the aqueous solution evaporated to dryness, and the residue extracted with boiling absolute alcohol. The amino-acids insoluble in absolute alcohol weighed 23.47 gm. and by fractional crystallization yielded 15.57 gm. of leucine. The remainder of this fraction was united with the mother liquors from the leucine obtained from Fraction II.

Fraction II yielded 17.66 gm. of amino-acids insoluble in alcohol, from which 13.10 gm. of leucine were separated by fractional crystallization.

The solutions from which the leucine had separated, together with the more soluble part of Fraction I, and also the mixed acids recovered from the alcohol and ether, as previously stated, by fractional crystallization yielded 4.2 gm. of leucine, 9.74 gm. of alanine and 8.65 gm. of a mixture, probably largely valine, with some leucine and alanine. This mixture was destroyed by fire before it was further examined, hence no estimation of valine was made.

From the united alcoholic extracts of the amino-acids from Fractions I and II the proline was isolated as the copper salt, the weight of which, when dried at 110°, was equal to 16.6 gm. of proline.

There were thus obtained from Fractions I and II 18.07 gm. of alanine, or 9.79 per cent; 34.73 gm. of leucine, or 18.82 per cent, and 16.6 gm. of proline, or 8.99 per cent of the zein.

Fraction III which weighed only 3.86 gm., when saponified with baryta and worked up in the usual way, yielded no copper aspartate and was lost before anything definite was obtained from it. It probably consisted chiefly of leucine ester.

The distillation residue was also lost, hence no determinations of phenylalanine, aspartic or glutaminic acid were made.

The figures for leucine and proline agree closely with the higher results previously obtained. The amount of alanine however is much greater than that found in any of the earlier analyses and shows plainly the necessity of using care to recover all esters that may be carried off with the ether or alcohol distilled from the free esters. In the following table we give the results of all the determinations of alanine, leucine and proline from zein which have thus far been made in this laboratory.

	Osborne and Clapp.	Osborne and Jones.			Osborne and Liddle.
		1	2	3	
Alanine . . .	2.23	....	6.08	3.42	9.79
Leucine . . .	18.30	19.55	18.30	17.76	18.82
Proline . . .	6.53	8.23	9.04	7.11	899

Since all the determinations of the mono-amino-acids are to be considered as distinctly lower than the quantities actually yielded by the protein, it seems reasonable to accept the highest figures obtained, as most nearly representing the actual quantity of these substances present in the hydrolysis solution, provided care is taken to weigh only pure products when making such determinations.

We may therefore consider that the following table more nearly represents the actual proportion of the products of hydrolysis of zein than do any one of the separate analyses of this protein.

ANALYSIS OF ZEIN.

Glycocoll	0.00	Serine	1.02
Alanine	9.79	Tyrosine	3.55
Valine	1.88	Arginine	1.55
Leucine	19.55	Histidine	0.82
Proline	9.04	Lysine	0.00
Phenylalanine	6.55	Tryptophane	0.00
Aspartic acid	1.71	Ammonia	3 64
Glutaminic acid	26.17	Carbohydrate	0.00
Total			85.27

A CONSIDERATION OF THE SOURCES OF LOSS IN ANALYZING THE PRODUCTS OF PROTEIN HYDROLYSIS.

By THOMAS B. OSBORNE and D: BREESE JONES.

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IT is plain from all that is said and written that there is a widespread feeling of disappointment in respect to the progress that has been made within the last few years in our knowledge of the chemistry of proteins.

The high hopes raised by the analytical methods introduced by Emil Fischer appear to have led to the assumption that we should soon know practically all of the constituents of the more important proteins, and that no considerable part of these would long remain the subject of doubt and conjecture. Although it was generally realized that much time and labor would be required to obtain a knowledge of the actual combinations of the many primary building stones within the molecules of these proteins, nevertheless, the immense importance to physiology of a reasonably complete qualitative knowledge of their make-up, and an approximate knowledge of the proportions in which the several amino-acids are contained in them, led to the expectation that much light was about to be shed on multitudes of obscure problems. It is not surprising therefore to find that many point to the large deficiency shown by even the most successful protein analyses, and ask what it is which makes up the large undetermined part? Without a knowledge of this unknown moiety we are unable to use these analyses for many of the purposes for which they were originally intended, and consequently their value is greatly curtailed.

The development of almost every analytical method has been slow, and the working out of the conditions necessary for its success-

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[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

ful application has required long and patient study of the minor details of the various steps involved. It is only natural to expect that an analytical method, which is probably more complicated than any heretofore used, should at first yield results which would be much inferior to those which might later be obtained after knowledge and experience had been acquired in applying it many times. In the following pages we discuss the various sources of loss encountered in making protein analyses, and give such information respecting their nature and extent as we have thus far been able to obtain.

In carrying out an analysis of the mixture of mono-amino-acids, according to the now well-known method of Emil Fischer, there are several steps which must receive especial attention if the best results are to be obtained.

First the *hydrolysis* must be complete, that is, the protein must be so far decomposed into amino-acids that no combinations of these with one another remain in the solution.

Second the *esterification* of the amino-acids which are to be determined in the mixture of esters must be complete.

Third the *distillation* of these esters must not involve losses through incomplete distillation or incomplete condensation or destructive secondary decomposition.

Fourth the *separation* of the amino-acids regenerated from the esters must be effected without loss.

That, with the exception of the first, none of these requirements are fulfilled, was pointed out by Fischer himself in describing his method. To what extent this failure to fulfil these requirements affects the results of the analyses, has not yet been shown.

HYDROLYSIS.

1. *Complete hydrolytic decomposition of the protein.*

In order to completely hydrolyze a protein it is obvious that all of it should first be brought into solution. This is accomplished in some cases with difficulty, for the concentrated acid usually converts the protein into a gelatinous mass which dissolves gradually, leaving more or less sticking to the sides and neck of the flask, and this subsequently passes into solution slowly. It is difficult to determine when solution is complete for, even after the most prolonged hydrolysis, a certain small amount of substance remains in that insoluble condition, which is commonly designated melanin or humin. As an admixture of undissolved protein cannot be detected by the eye, it is important to apply the biuret test to the undissolved substance, so as to be sure that none of the so-called melanin consists, in fact, of protein. Even if no biuret reaction is obtained, it is by no means certain that the hydrolysis is complete, for resistant products may be formed which are insoluble in acid solutions, but which, when subjected to more energetic action of acids, can be decomposed into free amino-acids.

Thus we have found that, when gliadin was digested for six and one-half hours on the water-bath with 25 per cent sulphuric acid and then boiled in an oil-bath for eight hours, a relatively large insoluble residue was suspended in the solution. This was thoroughly washed with hot water and with alcohol and then boiled with 20 per cent hydrochloric acid for six hours. After filtering out the smaller amount of black insoluble residue the solution was cleared with bone black and examined for amino-acids. As it gave only a very slight Millon's reaction, and no tyrosine could be separated, it is plain that the insoluble substance remaining, after boiling with sulphuric acid, did not consist of unaltered gliadin. From this solution much glutaminic acid, and a not inconsiderable quantity of cystine, were isolated in a pure state. Evidence of leucine, relatively much proline, the dipeptide of proline and phenylalanine and other amino-acids were also obtained but, owing to the small quantity of substance and the number of amino-acids, these could not be separated in a pure enough condition for strict chemical identification.

The results obtained, however, were such as to leave no question that the relative proportion of these amino-acids was very different from that in gliadin, and that the substance was a polypeptide-like combination, or a mixture of such combinations more difficult to hydrolyze than the great bulk of the protein.

Fischer and Abderhalden[2] have shown that a part of certain proteins resists the prolonged action of trypsin, but that on boiling this with acid it is converted into amino-acids. Levene and Beatty[3] state that, even after boiling gelatin with 25 per cent sulphuric acid for twelve hours, substances of peptide character still remain. More recently Abderhalden and Brahm[4] obtained from silk an even more resistant product than that which we obtained from gliadin, for this could be converted into amino-acids only by heating with alkalies.

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[2] Fischer and Abderhalden: Zeitschrift für physiologische Chemie, 1903, xxxix, pp. 81–94.

Another long known example is antialbumid, for, according to our present knowledge of protein hydrolysis, this cannot be anything else than a resistant combination of amino-acids, the true character of which has not heretofore been recognized.[5] We thus have evidence that many different products which are *insoluble* in acid and are difficult to decompose may be formed during the process of hydrolyzing a protein.

That combinations of amino-acids easily *soluble* in acids may occur, which are very resistant to hydrolysis, is shown by the behavior of the prolyl-phenylalanine which Osborne and Clapp isolated from the products obtained by heating gliadin for six hours on the water-bath and then boiling for thirteen hours in an oil-bath with 25 per cent sulphuric acid.

As many polypeptides give no biuret reaction, the failure of this test, when applied to the solution, or to the insoluble substance which results after boiling with strong acids, is by no means a guarantee that the hydrolysis has been complete.

It is thus evident that loss may occur in the protein analysis through incomplete hydrolysis, and that at present we have no means for ascertaining when this process has been carried to its end.

The only way in which such losses can be reduced, if not certainly avoided, is by boiling with acids for a much longer time than has heretofore been the practice.

As our present experience shows that sulphuric acid is a less energetic hydrolytic agent than hydrochloric, the latter should always be used when maximum yields are desired.

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[3] LEVENE and BEATTY: Zeitschrift für physiologische Chemie, 1906, xlix, p. 247.

[4] ABDERHALDEN and BRAHM: Zeitschrift für physiologische Chemie, 1909, lxi, p. 256.

[5] Cf. CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291.

The facts just stated make it probable that more or less of the deficiency of such protein analyses, as have heretofore been made, may have been caused by incomplete hydrolysis.

2. *Formation of Melanin or Humin.*

It has long been known that a small amount of a black amorphous product, which is insoluble in the acid solution, is formed from proteins by the prolonged action of hot acids. This substance has been the subject of study, as it has been supposed to have relations with some of the black coloring matters occurring in nature which are known as melanins.[6]

There can be no doubt that this substance is a mixture of secondary decomposition products formed from different constituents of the protein by the action of acids, for some of these, such as tryptophane, histidine, and carbohydrates, are known to yield colored products under such conditions. That the " melanin " originates chiefly from these substances is indicated by the fact that zein, which gives no reaction for tryptophane or carbohydrate, and yields only a very small amount of histidine, gives rise to the merest trace of humin when subjected to prolonged hydrolysis with acids.

In view of what has just been stated it is clear that the so-called melanin, which results by acid hydrolysis from proteins, has a very remote relation, if any, to the naturally occurring black pigments to which the name melanin was first applied, and that it would be much better to return to the older and more indefinite name of humin or humus.

The amount of humin which separates as a flocculent residue from the hydrolysis solution of proteins seems to differ not only with the protein but also with the conditions of hydrolysis. In general it forms from 1 to 2 per cent of the protein, but accurate data are not yet available.

As the *insoluble* humin is a product of secondary decomposition of constituents of the protein it is certain that the quantity of the substances from which it originates is greater than that of the humin itself. How much substance also contributes to the formation of the *soluble* coloring matters, which always darken the hydrolysis solution, cannot be even approximately estimated.

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[6] Cf. SCHMIEDEBERG: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xxxix, p. 65; also CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291; also SAMUELY: Beiträge zur chemischen Physiologie und Pathologie, 1902, ii, p. 355.

It is evident that a loss is thus caused in the analysis which in most cases must amount to at least several per cent of the protein analyzed.

SEPARATION OF GLUTAMINIC ACID.

It seems to be generally assumed that glutaminic acid can be determined with considerable accuracy, and the results of such determinations have been used many times for comparing proteins with one another and for detecting changes in them under various conditions.

Our experience has shown us that much uncertainty still attaches to these determinations, especially in the case of some of the proteins which have been already analyzed.

As no means are available for directly proving the completeness of the separation, conclusions can be based only on indirect and unconvincing evidence. That agreement between two or more determinations is not to be accepted as indicating a complete separation, has been repeatedly shown by our experience. Thus several determinations of glutaminic acid in zein, which in each case yielded approximately 18 per cent, were later found to be at least 8 per cent too low, for a reason that will soon be given. Similarly, while Osborne and Gilbert[7] formerly isolated from edestin 14 per cent of glutaminic acid, and Abderhalden 14.5 (communicated to the writer by letter), it has been shown in a previous paper[8] that at least 18.74 per cent is yielded by this protein.

On the other hand, as previously pointed out[9] in the case of gliadin, by applying the ester method to the solution from which the greater part of the glutaminic acid had been separated as hydrochloride, a further quantity was obtained, which agreed almost exactly with the largest previously obtained by direct separation as hydrochloride, and it was concluded that, in this case, the result was probably very nearly correct. We have, however, since isolated, after thirty-eight and one-half hours hydrolysis, 42.98 per cent, or 5.48 per cent more than the highest result previously obtained after about eighteen hours hydrolysis.

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[7] OSBORNE and GILBERT: This journal, 1906, xv, pp. 333-356.

[8] OSBORNE and LIDDLE: This journal, 1910, xxvi, p. 295.

[9] OSBORNE, LEAVENWORTH and BRAUTLECHT: This journal, 1908, xxiii, p. 180.

Similarly after thirty-eight and one-half hours hydrolysis we have also obtained, by direct separation as hydrochloride, much higher results for rye gliadin and hordein, than formerly after fifteen to eighteen hours hydrolysis, namely, rye gliadin 37.8 per cent, and hordein 43.19 per cent, instead of 33.81 per cent and 36.35 per cent respectively.

Whether our higher results were actually due to the longer hydrolysis, or to more experience in conducting the process of separation than when the earlier determinations were made, is not wholly clear. In the case of hordein the longer hydrolysis may not have contributed to the higher result, for Kleinschmitt,[10] who boiled his solution for only five hours, obtained almost as much as we did after thirty-eight and one-half hours, namely 41.32 per cent. It is possible that the difference between the earlier and the present result is due to the fact that, in the first determination, ammonia was removed by treating the solution of the glutaminic acid hydrochloride with an excess of baryta, and passing carbonic acid through the solution for some time. Under these conditions any barium glutaminate that had separated may have been filtered out with the barium carbonate and lost, for at the time this determination was made the danger of such a loss was not recognized. This criticism does not apply to the other determinations, as ammonia was removed from the glutaminic acid hydrochloride in the above way only in the case of hordein.

Losses may also occur through incomplete crystallization of the glutaminic acid hydrochloride, and may not be recognized unless the solution is afterwards esterified, and the esters and distillation residue carefully examined for glutaminic acid. No result can, therefore, be accepted as probably showing the approximate quantity of glutaminic acid yielded by any protein, until it has been controlled by the ester method.

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[10] KLEINSCHMITT: Zeitschrift für physiologische Chemie, 1907, liv, pp. 110–118.

There are some proteins from which, as has already been pointed out in papers from this laboratory, the glutaminic acid can be obtained only with great difficulty, for they seem to yield a combination of amino-acids which prevents the separation of a large part of the glutaminic acid hydrochloride. In such cases it is difficult to obtain uniform results, since the separation of the hydrochloride depends on a proper adjustment of conditions, which can only be obtained by accident.

Thus Osborne and Gilbert,[11] after repeated trials, isolated from wheat leucosin a maximum of 5.72 per cent of glutaminic acid, whereas Osborne and Clapp[12] obtained from a much larger quantity of this protein 3.85 per cent by direct separation and 2.88 per cent more from the esters, making in all 6.73 per cent.

From pea legumelin Osborne and Heyl[13] obtained 10.52 per cent of glutaminic acid as the hydrochloride, and 2.44 per cent more from the esters, a total of 12.96 per cent, whereas a separate, direct determination, carried out with especial care and with the expectation of obtaining a larger yield, gave only 5.76 per cent of glutaminic acid.

Some of the other proteins from leguminous seeds present similar difficulties, and it is still uncertain just how much glutaminic acid is actually yielded by them. Whether or not this trouble is caused, in part or in whole, by the character of the other decomposition products, or by incomplete hydrolysis, remains to be shown by further study.

As already stated, it is difficult to obtain satisfactory determinations of glutaminic acid for zein, the reason for which, has been found in the unusually large amount of leucine yielded by this protein. When the hydrolysis solution is saturated with hydrochloric acid and cooled with ice it solidifies completely, and no point can be found at which the glutaminic acid hydrochloride separates, leaving the other amino-acids in solution. In one case we obtained a separation of hydrochlorides which, if all were glutaminic acid hydrochloride, would be equal to over 30 per cent of the zein. Examination of this showed it to contain a large proportion of leucine hydrochloride from which the glutaminic acid was separated with great difficulty. Although by reprecipitation with hydrochloric acid a large part could easily be separated pure, the leucine prevented complete crystallization, so that it was necessary to convert the mixture contained in the mother liquor into the free amino-acids and the glutaminic acid into its acid sodium salt by neutralizing to litmus, and then to allow the free leucine to crystallize out. When leucine had thus been separated, a further quantity of glutaminic acid was isolated as the hydrochloride. That leucine hydrochloride may thus crystallize together with glutaminic acid hydrochloride does not appear to have been before recognized.

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[11] Osborne and Gilbert: This journal, 1906, xv, pp. 333–356.

[12] Osborne and Clapp: *Ibid.*, 1906, xvii, pp. 231–265.

[13] Osborne and Heyl: Journal of biological chemistry, 1908, v, pp. 197–205.

ESTERIFICATION.

If only 75 per cent of the amino-acids are converted into esters at each operation, the proportion remaining unesterified after the second operation would form only 6.25 per cent of the amino-acids originally present. As usually 40 to 50 per cent of the decomposition products of the proteins are obtained otherwise than through their esters, the total losses arising from incomplete esterification need not necessarily be great.

That in practice the esterification is as complete as here assumed, is indicated by the small amount of amino-acids which have been recovered after removing the esters formed by a second esterification. In the several cases in which we have weighed these acids about 6 per cent of the protein was thus recovered. Since mechanical losses of not inconsiderable extent are involved in the process employed in separating these unesterified acids, the esterification is in fact not as complete as indicated by the amount of unesterified acids which are recovered.

The conditions under which the water formed during the process of esterifying by Fischer's method is removed, are such, that there is great danger of decomposing esters already formed, unless sufficient alcohol is added to carry off with its vapors all of the water present. If this is not the case the solution, during evaporation, becomes gradually richer in water and consequently more or less of the esters may be decomposed.

As it is difficult to provide with certainty against this danger, it is possible that, in many cases, losses have arisen from this cause which have contributed to the low summation of many of the analyses heretofore made. This danger can be easily avoided by employing the method used by Phelps and Tillotson for esterifying organic acids, for by this process the water is constantly removed as fast as it is formed and, theoretically, a maximum yield of esters should be sooner obtained than by Fischer's method. That a higher yield of esters can be obtained by Phelps and Tillotson's method, is indicated by a comparison of the analyses of zein made by these two methods, which have recently been described in a paper from this laboratory.[13a]

This comparison indicates that a part of the deficiency of previously made protein analyses is to be attributed to incomplete esterification, but to what extent, cannot be determined from the data now available.

Losses also occur from incomplete extraction of the liberated esters when these are shaken out with ether, but these are reduced to a comparatively small amount if care is taken when extracting the much smaller quantity of material which remains after the second esterification. Losses may also occur, and sometimes doubtless have occurred, during the drying of the esters over sodium sulphate, for we have recently found that the sodium sulphate, after carefully washing with ether, in some cases contained a notable quantity of nitrogen while in other cases it contained none.

Whether or not any of the esters of the *mono*-amino-acids contributed to the nitrogen thus found has not yet been determined. The possibility of a loss during this process is one that should not be overlooked and deserves further study.

Losses of considerable magnitude take place in removing the ether from the esters previous to distillation under diminished pressure. That glycocoll ester is carried over with the ether has been known for a long time and it is the usual practice to recover this from the distilled ether but that other esters are also carried over in considerable quantity does not appear to have been generally recognized, although Fischer has stated that a little alanine might be thus lost.

In a recent paper[14] from the laboratory such data as have been thus far obtained are given and it was shown that the amount of alanine, as well as other amino-acids, thus lost was relatively considerable.

[13a] OSBORNE and JONES: This journal, 1910, xxvi, p. 212.

[14] OSBORNE and LIDDLE: This journal, 1910, xxvi, p. 295.

THE ACTUAL EXTENT OF THE LOSSES INCURRED IN ANALYZING A PROTEIN.

From a consideration of all of the facts thus set forth it is clear that many causes of loss are connected with our present methods of analyzing the products of protein hydrolysis. We have consequently endeavored to obtain more definite information on this question, first by analyzing a protein with especial reference to securing as high results as possible and to gaining further knowledge of the amount and character of those products which cannot be separated into definite substances and, second, by analyzing by the same method and with the same care a mixture of pure amino-acids made in the same proportion as found in the protein analyzed.

THE ANALYSIS OF THE PROTEIN.

The protein selected for this analysis was zein because it yields no tryptophane or carbohydrate, neither of which can be quantitatively determined in the mixture of decomposition products with any approach to accuracy. It furthermore yields no lysine and less arginine and histidine than any other known protein and as it therefore yields a correspondingly larger proportion of mono-amino-acids it is well suited for our present purposes.

The results of this analysis have already been published in a recent paper[15] in which will be found a detailed account of the conditions under which the analysis was conducted. These, therefore, need not be repeated here, for it is our present intention to consider the probable quantity of the recognized decomposition products originally yielded by the zein, and thus obtain some idea of the probable amount of products of still unknown nature which this protein yields.

In this analysis of zein we isolated at the outset 135 gm. of crude hydrochlorides which contained 13.22 gm. of ammonium chloride. Deducting this latter we have 121.78 gm. of substance which consisted chiefly of glutaminic acid hydrochloride, together with a little leucine hydrochloride. It will not involve any serious error to assume that 80 per cent of amino-acids were present in this 121.78 gm. of substance, or 97.42 gm. Arginine, histidine and ammonia were not included in any of the weighed fractions derived from the esters unless, possibly, the distillation residues may have contained a small amount of them. Their absence from this residue may, however, for our present purposes, be assumed. On the basis of these assumptions we have the figures given in the following table.

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[15] OSBORNE and JONES: This journal, 1910, xxvi, p. 212.

THE PRODUCTS OF HYDROLYSIS OF 367.35 GRAMS OF ZEIN.

Substances taken.	Calculated.	
	As free amino-acids.	As radicals in peptide union.
	gm.	gm.
Free amino-acids, chiefly glutaminic acid, separated as hydrochlorides	97.42	74.22
Valine and alanine	33.12	27.26
Leucine	63.47	54.76
Proline	33.20	28.01
Phenylalanine	22.95	20.45
Aspartic acid	6.35	4.63
Glutaminic acid (from esters)	23.35	17.62
Serine	3.76	3.12
Tyrosine	13.00	11.70
Arginine	4.96	4.46
Histidine	3.01	2.66
Ammonia	13.37	12.58
Unesterified residue of undertermined nature .	12.96	11.02
Crystalline mixtures of undertermined nature .	16.12	13.70
Undistilled residues, chiefly decomposition products of the esters	34.00	28.90
Total	381.04	315.09

The total weight of the recovered products, which is given in the first column, is greater than that of the zein hydrolyzed, because free amino-acids were weighed, whereas, within the protein these were combined with one another as radicals, probably in peptide union. We therefore give in the second column the calculated weights of these radicals, those for the dibasic acids being also calculated as combined through one carboxyl group with ammonia.

The quantity of unidentified amino-acids found in the "Unesterified residue" and also in the "Crystalline mixtures" is estimated on the assumption that 80 per cent of their quantity represents approximately the weight of the corresponding radicals originally contained in the zein. We have but little definite information as to the nature of the "Distillation residues" but we have assumed that 80 per cent of these approximately represents the weight of radicals from which they originated.

Such a calculation of course must not be considered to *accurately* represent the actual proportion of the several radicals contained in the zein, for it is based on assumptions which, although supported by indirect evidence, are as yet by no means proved. The sum of these figures is, however, far less misleading in estimating the probable total of substances accounted for in this analysis, than that of the free amino-acids. We, therefore, consider ourselves justified in making this calculation, as it is our purpose to indicate approximately the amount of substance which had not been located by this analysis.

The total amount of the 367.35 gm. of zein thus located is 315.09 gm. or 85.8 per cent of the zein. Of this 315.09 gm., 24.72 gm. (11.02 + 13.70), or 6.7 per cent, consists of mixtures composed chiefly of the above enumerated amino-acids which could not be separated, and 28.9 gm., or 7.8 per cent, of the substances which constitute the undistilled residues. We thus have 14.5 per cent of the zein as substance which has not been positively identified. If this 14.5 per cent of unidentified substances is deducted from the 85.8 per cent of total substances weighed and located, we find that 71.3 per cent of the zein has been actually accounted for in percentages of definite substances. Of the 28.7 per cent not assigned to specific amino-acids, 6.7 per cent consists of crystalline mixtures of products not pure enough to permit of their strict chemical identification, but which were unquestionably mixtures of the same amino-acids which had been identified as products of hydrolysis of zein. We thus find that at least 78 per cent of the zein consists of amino-acids, which are now known to be decomposition products of proteins; thus leaving only 22 per cent which may possibly belong to substances of still unknown nature.

A part of this deficit consists of substances contained in the esters distilling over with the higher boiling fraction which contains the esters of phenylalanine, aspartic and glutaminic acids and serine. After separating these amino-acids a considerable quantity of substance always remains, from which serine separates, if at all, slowly and probably very incompletely. That this substance contains secondary decomposition products of the esters formed during distillation, is indicated by the fact that the esters obtained from a mixture containing the same amino-acids, and in the same proportion as those obtained from zein (see page 320) yielded a corresponding product in approximately the same amount. Thus 33.17 gm. of the esters from this mixture, distilling between 105° and 150° at 0.6 mm., yielded 9.13 gm. of phenylalanine, 2.55 gm. of aspartic acid, and 3.91 gm. of glutaminic acid. The weight of the esters corresponding to these amino-acids is 17.53 gm., leaving 15.64 gm. or 4.8 per cent of the mixture unaccounted for.

In the analysis of zein a fraction of the esters distilling between 105° and 150° weighed 50.56 gm. This yielded 17.95 gm. of phenylalanine, 8.99 gm. of glutaminic acid, and 3.25 gm. of aspartic acid, equal to 35.25 gm. of esters, thus leaving 15.31 gm. or 4.2 per cent of the zein unaccounted for. Similar products have been obtained from the esters of all the proteins analyzed, and it has always seemed possible that some new protein decomposition product would be sooner or later separated from this substance. A little serine can commonly be separated from it, and it is possible that a part of this product obtained from zein may have been serine ester, but as the mixture of amino-acids contained only 2 gm. of serine, this could have contributed but little to the total of 15.64 gm.

The total amount of this unidentified substance in the analysis of zein can be estimated as follows. The distillate obtained above 105° weighed 95.27 gm. The weight of the esters corresponding to the free acids isolated from these esters was 68.94 gm. leaving a difference of 26.23 gm. of the distillate to be accounted for, or 7.14 per cent of the zein. Assuming that at the most 80 per cent of this was originally contained in the zein we have 21.06 gm. or 5.7 per cent of the zein.

We therefore have one-fourth of the 22 per cent deficit located in this undetermined part of the upper fractions. Of the remaining 16.3 per cent a part is doubtless to be assigned to cystine, but if all the sulphur of zein orginally belongs to a cystine complex, this could not exceed 2.5 per cent. That a part of the unknown substance of the upper fraction may be derived from decomposition of cystine is indicated by the fact that sulphur compounds are frequently present in the decomposition products condensed by liquid air during the distillation of the upper fractions, and in that case a deduction for cystine based on the sulphur content of the protein cannot be made without counting at least a part of the cystine twice.

A further part of this 16.3 per cent of unidentified substance is contained in the distillation residues which in this analysis weighed 34 gm., equal to nearly 10 per cent of the zein. Little is known of the real nature of this residue, but from the fact that alcohol is evolved in considerable quantity during the distillation it seems highly probable that a not inconsiderable part consists of condensation products of the esters due to loss of alcohol during the distillation. That this is the case is evidenced by the formation of similar residues during the distillation of the esters made from the pure amino-acids, as will soon be shown. How large a part of these residues are thus formed cannot be definitely stated, for a part of the alcohol which first comes over undoubtedly is alcohol retained by the esters, and not liberated by condensation of the esters. The not inconsiderable quantity of alcohol coming off during the later stages of the distillation, however, without question, results from the latter cause, and indicates a considerable loss of amino-acids which are already known to be products of hydrolysis of the protein.

If the amount of nitrogen contained in the different radicals given in the second column of the table is calculated, that in the substances of unknown nature being assumed to be 12 per cent, or the same as in the leucine radical, we find that 51.05 gm. or 86.8 per cent of the total is thus accounted for, or approximately the same proportion of the total protein as is accounted for by the products enumerated in the table. As 52.26 gm. of the zein and 7.73 gm. of the nitrogen is thus unaccounted for, the mean nitrogen content of the unaccounted for part is 14.8 per cent.

ANALYSIS OF THE MIXTURE OF AMINO-ACIDS.

The next question that naturally suggests itself is, how great is the actual yield when a mixture of weighed quantities of these same amino-acids is subjected to the same treatment? As an answer to this question would shed much light on the probable quantity of the different mono-amino-acids which are actually contained in the hydrolysis solution, we made a mixture of the different amino-acids in approximately the same amount as we obtained them from zein. The amino-acids used in this mixture were very carefully prepared and then subjected to a rigid test for their purity.

MIXTURE OF AMINO-ACIDS ANALYZED.

Glutaminic acid hydrochloride	150.0 gm. =	120.0 gm. free acid
Alanine		16.0 " " "
Valine		4.0 " " "
Leucine		85.0 " " "
Proline ethyl ester	38.6 gm. =	31.0 " " "
Aspartic acid		6.0 " " "
Phenylalanine hydrochloride	31.7 gm. =	26.0 " " "
Tyrosine		16.0 " " "
Arginine methyl ester hydrochloride	8.2 gm. =	5.5 " " "
Serine		2.0 " " "
Histidine dichloride	3.0 gm. =	2.0 " " "
Ammonium chloride	45.0 gm. =	14.3 NH_3
Total		326.0 gm.

This mixture was dissolved in dilute hydrochloric acid by heating on a water-bath, and the solution then evaporated at a low pressure and temperature till crystallization began. Alcohol was then gradually added, and the evaporation continued until almost all the water was removed, and the hydrochlorides of the acids were dissolved in strong alcohol.

First esterification. — The solution of the hydrochlorides of the amino-acids was evaporated to a thick syrup under strongly diminished pressure, 20 c.c. of alcohol saturated with hydrochloric acid added, and 35 gm. of zinc chloride. The vapors of 1800 c.c. of absolute alcohol containing 36 c.c. of alcoholic hydrochloric acid were then passed through the solution for five hours, during which time the mixture was kept at 103° to 107°.

The esters were liberated with sodium hydroxide and potassium carbonate in the usual way, and their solution in ether was dried over sodium sulphate for one week. After removing the ether by distillation at atmospheric pressure the esters which remained weighed 258 gm. equal to 79 per cent of the mixture.

The esters were distilled as follows:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I (*a*)	80°	0.7 mm.	58.65 gm.
(*b*)	105°	0.55 "	42.08 "
	Condensed by liquid air	...	13.00 "
II	150°	0.6 mm.	26.00 "
	Condensed by liquid air	...	15.00 "
	Distillation residue	...	93.00 "
Total	. . . .		247.73 gm.

The distillation of these esters proceeded throughout in the manner characteristic of the esters obtained from the products of hydrolysis of proteins. At a temperature of about 120° of the oil-bath, evidence of more or less decomposition appeared in the form of vapors, which were condensed by the liquid air in just the same way as we have usually observed when distilling the esters from proteins.

The undistilled residue presented to the eye all the characteristics of such residues, and its subsequent examination indicated no unusual properties.

Fraction I, together with the products condensed by liquid air, was saponified by boiling with water in the usual way, and the solution evaporated to a crystalline residue, which was freed from water by repeated evaporation with absolute alcohol under diminished pressure, and then extracted with absolute alcohol until all the proline was removed.

The amino-acids insoluble in alcohol weighed 63.3 gm., and by fractional crystallization yielded 53.18 gm. of leucine, 7.34 gm. alanine, and 1.40 gm. of valine; the loss in undetermined mixtures being 1.38 gm. The leucine and valine were separated by the lead salts.

The alcoholic extract was freed from all substances not readily soluble in absolute alcohol, and the dissolved proline converted into the copper salt. Of the l-proline, 14.82 gm. were obtained, and of the r-proline 4.72 gm.

Fraction II, by the customary treatment, yielded 7.37 gm. of phenylalanine, 2.55 gm. of aspartic acid, and 2.93 gm. of glutaminic acid.

A syrupy residue remained from which nothing definite could be isolated. After long drying over sulphuric acid this weighed 5.88 gm., and contained 0.4557 gm. nitrogen, of which 0.0490 gm. was as ammonia.

The distillation residue was treated with five volumes of water, and shaken out with ether in the same way as Fraction II. From the ether solution 0.74 gm. of an ill-defined resinous substance was isolated and 8.27 gm. of phenylalanine. The aqueous solution, when saponified with baryta yielded 7.56 gm of tyrosine, 47.18 gm. of glutaminic acid and a syrup which, after long drying, weighed 5.4 gm. and contained 0.5110 gm. nitrogen of which 0.0119 gm. was as ammonia. We thus recovered from the distillation residue 63 gm. of well defined products which are equivalent to 74.88 gm. of ester. This leaves 18.12 gm. of substance unaccounted for or 5.5 per cent of the original mixtures of amino-acids.

Second esterification. — The unesterified substances which remained after the first shaking out with ether were freed from inorganic salts and water, by repeatedly evaporating with alcoholic hydrochloric acid. To the residual syrup thus obtained 20 c.c. of alcoholic hydrochloric acid, and 10 gm. of zinc chloride were added and the vapors of 1000 c.c. of absolute alcohol, containing 20 c.c. of alcohol saturated with hydrochloric acid, were passed through the solution during six hours. The esters were shaken out with ether as usual. After removing the ether the crude esters weighed 108 gm. equal to 33.1 per cent of the original mixture of amino-acids. The total quantity of esters thus obtained was, therefore, 112.1 per cent of the mixture. These esters, however, contained a little ether and alcohol so that the actual yield of esters was not quite so large.

The second crop of esters was distilled with the following results:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	110°	0.85 mm.	25.43 gm.
	Condensed in liquid air	...	10.00 "
II	150°	0.95 "	7.17 "
	Condensed to liquid air	...	1.29 "
	Undistilled residue	...	52.00 "
Total			95.89 gm.

The loss of 12.11 gm. indicated by the total of the products of distillation, is chiefly ether which was not condensed at the beginning of the distillation.

Fraction I, together with the substance condensed by liquid air, was saponified by boiling with water, the solution evaporated to dryness under low pressure, and the residue freed from water by evaporating several times with absolute alcohol. The residue insoluble in alcohol weighed 17.77 gm., and by systematic fractional crystallization yielded 15.72 gm. of leucine, 0.24 gm. of valine and 1.40 gm. of a mixture from which no definite product could be obtained. As this contained 49.4 per cent of carbon and 9.34 per cent of hydrogen it doubtless contained some alanine. The total losses in these separations, as determined by difference, were 1.81 gm.

The alcoholic extract yielded 3.02 gm. of proline which was weighed as the copper salt.

Fraction II yielded 1.76 gm. of phenylalanine, no aspartic acid, 0.98 gm. of glutaminic acid, and a syrup, which, after long drying over sulphuric acid, weighed 3.47 gm. and contained 0.2121 gm. of nitrogen of which 0.0098 gm. was as ammonia.

The distillation residue was treated with 300 c.c. of water and shaken out with an equal volume of ether, as Fischer directs for the treatment of esters distilling at the higher temperatures. From the ether solution 0.67 gm. of phenylalanine was obtained, and from the aqueous solution, after saponifying with baryta, 32.17 gm. of glutaminic acid, and a syrup of unknown nature, which after prolonged drying weighed 6.33 gm. and contained 0.5900 gm. of nitrogen, of which 0.0119 gm. was as ammonia.

The esters corresponding to the phenylalanine and glutaminic acid isolated from this residue correspond to 40.31 gm., which if deducted from the weight of the residue, 52 gm., leaves 11.69 gm. or 3.6 per cent of the total weight of the mixture of amino-acids.

Adding these to the corresponding quantities obtained from the products of the first esterification, we have a total of 29.81 gm. of unidentified substances in the undistilled residue, or 9.1 per cent of the original mixture. This proportion corresponds closely with that obtained from the esters from zein, namely 34 gm. or 9.2 per cent.

The unesterified substances.—After the second esterification nearly all the inorganic salts were removed from the residue by repeated evaporation with alcoholic hydrochloric acid. The solution was then largely freed from hydrochloric acid by diluting with water and evaporating several times under diminished pressure. The syrupy residue was then dissolved in 3000 c.c. of 5 per cent sulphuric acid, and treated with a solution of phosphotungstic acid as long as a precipitate formed, which was filtered off the next day and decomposed with baryta. The basic amino-acids were then determined according to the method of Kossel and Patten in the solution freed from baryta and sulphuric acid, and 0.80 gm. of histidine and 3.57 gm. of arginine found. The filtrate from the phosphotungstic acid precipitate was freed from this acid, and also from sulphuric acid, concentrated to a small volume, and the remaining alkali-chlorides removed almost completely with alcoholic hydrochloric acid. The rest of the chlorine was removed with silver sulphate and the solution evaporated to dryness. The crystalline residue weighed 8.0 gm. By fractional crystallization this yielded 0.41 gm. tyrosine and 0.46 gm. leucine. No serine or any other definite substance could be isolated from the remainder.

In the following table we give the weights of the different amino-acids obtained from each esterification and the percentage of each recovered.

These figures show plainly that by our present methods of analyzing proteins, relatively large losses result, and leave no doubt that much of the deficit of the analyses heretofore made, consists of undetermined quantities of those amino-acids which are already known to be decomposition products of the proteins.

In analyzing this mixture, less than one half of the alanine was recovered, which is probably largely due to losses caused by volatilization of the ester during the removal of the ether. Some loss also occurs in separating the free alanine in a condition fit to weigh, which is chiefly caused by its great solubility in water. Loss also results through incomplete separation from valine, as we have no method whereby this can be effected sharply. It is highly probable that less than half the alanine actually present has heretofore been isolated from proteins, except from those which, like silk fibroin, yield an unusually large amount.

The proportion of valine recovered was even less than that of alanine, which is to be attributed, in part at least, to the much smaller quantity present. The difficulties presented in separating valine from alanine involve losses which, in this case, were probably sufficient to account for a considerable part of the deficiency. The loss incurred in separating valine from leucine was relatively small, for this separation was effected by the lead method of Levene and Van Slyke which yields satisfactory results. Determinations of valine, made simply by fractional crystallization, are unquestionably far too low, and are to be regarded as merely qualitative.

SUMMATION OF THE ANALYSIS OF THE MIXTURE OF PURE AMINO-ACIDS.

Amino-acids taken.		Recovered from			Recovered from		
		Esters I.	Esters II.	Total.	Esters. I.	Esters. II.	Total.
	gm.				per cent		
Alanine	16.00	7.34	0.00	7.34	45.88	0.00	45.88
Valine	4.00	1.40	0.24	1.64	35.00	6.00	41.00
Leucine	85.00	53.18	16.18	69.36	62.56	19.04	81.60
Proline	31.00	19.54	3.02	22.56	63.03	9.74	72.77
Phenylalanine	26.00	15.64	2.43	18.07	60.16	9.35	69.51
Aspartic acid	6.00	2.55	0.00	2.55	42.50	0.00	42.50
Glutaminic acid	120.20	50.11	33.15	83.26	41.76	27.63	69.39
Tyrosine	16.00	7.56	0.41	7.97	47.25	2.56	49.81
Arginine	5.50	3.57	0.00	3.57	64.91		64.91
Histidine	2.04	0.80	0.00	0.80	39.21		39.21
Serine	2.00	0.00	0.00	0.00	0.00		0.00
Ammonia	14.31						
Total	328.05	161.69	55.43	217.12			66.17

The proportion of leucine which we recovered was greater than that of any of the other amino-acids. This is in agreement with our earlier experience in esterifying leucine alone, for we recovered 88.8 per cent of the original quantity after a single treatment.

In our three analyses of zein we obtained practically the same amount of leucine as in the earlier analysis made by Osborne and Clapp, according to Fischer's method of esterifying. It is probable that the proportion of leucine heretofore reported from such proteins as have been analyzed by the ester method, in most cases, more nearly represents the quantity actually yielded by them than do the figures given for any of the other mono-amino-acids obtained from the esters. The loss of nearly 20 per cent of the leucine, here shown, is probably chiefly caused by decomposition of its ester during distillation, as no other source of serious loss was apparent during the entire process.

The percentage of proline recovered was nearly as great as that of leucine. Analyses, in which Phelps and Tillotson's method of esterification was used, indicate that proline is more easily esterified in this way than by Fischer's method, for in each of three analyses, distinctly more was found than in the earlier analysis of Osborne and Clapp. The losses which occur, in thus isolating proline, are probably largely caused by a partial decomposition of its ester, since in distilling proline ester by itself, we found that a part did not distil over, even when the bath was at a high temperature and the pressure low. The undistilled residue was similar in appearance to that formed during the distillation of the esters from a protein.

It has generally been assumed that the amount of proline reported in most of the protein analyses is too high rather than too low, since it is very difficult to separate all of the other amino-acids by the methods usually employed. The result here given indicates that errors from this source do not compensate for the losses which occur from other causes, and that the results are, if anything, too low.

Of the phenylalanine practically 70 per cent was recovered, the loss probably being largely caused by decomposition of the esters during distillation.

The amount of aspartic acid that was recovered is less than one-half of that actually present. There are many ways in which loss may occur in separating aspartic acid, for some may be lost through decomposition of the esters in distilling, some through incomplete saponification with the formation of the half ester, and some through incomplete crystallization of the copper salt, which often separates very slowly from solutions containing other substances. As these sources of loss are difficult to avoid completely, it is not surprising.

where so little is originally present as in this mixture, that the percentage of loss should be as large as it is here.

In considering our analysis of zein, the results of which are given on page 316, it may be fairly assumed that the amount of tyrosine, arginine, histidine and ammonia are stated very nearly correctly. In regard to the accuracy of the figures for serine we know nothing whatever, but we have no conclusive evidence to show that any considerable quantity was not isolated.

The part of the glutaminic acid which was not separated as hydrochloride was subsequently largely obtained from the ester, and it may be assumed that the loss involved in separating this latter part, was at least as great, proportionally, as in the analysis of the known mixture of amino-acids. Assuming this to be true, and that the losses in separating alanine, valine, leucine, proline, phenylalanine and aspartic acid, were proportionally the same as in our analysis of the mixture, we have calculated the possible quantities of the several amino-acids originally yielded by the 367.35 gm. of zein hydrolyzed.

AS RADICALS IN PEPTIDE UNION.

	gm.		gm.
Free amino-acids, chiefly glutaminic acid, isolated as hydrochlorides	74.22	Aspartic acid	10.90
Valine and alanine	60.60	Glutaminic acid from esters	25.40
Leucine	67.11	Serine	3.12
Proline	38.47	Tyrosine	11.70
Phenylalanine	29.42	Arginine	4.46
		Histidine	2.66
		Ammonia	12.58
Total			340.64

In thus allowing for the analytical losses, the substances of unknown nature which appear in the table on page 316 as "Unesterified residue," "Crystalline mixture" and "Distillation residues" are omitted, for these unquestionably represent, to a large extent, losses similar to those involved in out analysis of the mixture of amino-acids. It is seen that by thus calculating the results of this analysis, that 340.64 gm. of the original 367.35 gm. are accounted for, or 92.7 per cent. Of the deficit of 7.3 per cent thus indicated, a part must be assigned to cystine, if its presence can be inferred from the 0.6 per cent of sulphur which zein contains, and a part, probably, to serine, which almost certainly was incompletely isolated.

Although these figures are based on assumptions that are not definitely proved, they justify the conclusion that a considerable part at least of the deficit shown by the analysis of zein consists of undetermined quantities of alanine, valine, leucine, proline, phenylalanine, aspartic acid, glutaminic acid and serine. The next step to a more complete knowledge of the decomposition products of the proteins is plainly to so far perfect our methods of analysis as to reduce the many losses now met with in the various stages of the analysis.

Those losses caused by incomplete hydrolysis can easily be reduced by longer boiling with acids. Losses incident to secondary decomposition during hydrolysis do not appear to involve any considerable quantity of the amino-acids which zein yields, but these can hardly be avoided so long as the hydrolyses are made with strong mineral acids. The losses connected with the esterification can be reduced to small amounts if care is taken in conducting the process. The greatest chance for improvement seems to lie in finding some method by which the esters can be liberated and dissolved in ether without mechanical losses.

The losses that occur during the distillation can be reduced by completing the process as rapidly as possible, but even so, these appear to be the most serious that are met with during the whole analysis. Thus in our analysis of the mixture of amino-acids, the amount of esters unaccounted for as definite substances in Fraction II and in the distillation residues, was equal to almost 14 per cent of the mixture. Assuming that 90 per cent of this is equivalent to the free acids originally contained in the mixture, we have at this stage of the process a loss of at least 12.5 per cent. The analysis of the mixture of amino-acids also shows that, in spite of nearly complete esterification, only 72.3 per cent of those amino-acids were recovered, which are separated by means of their esters, that is, of the 168 gm. consisting of alanine, valine, leucine, proline, phenylalanine, and aspartic acid, only 124.25 were recovered.

ON THE ALLEGED SPECIFIC ANÆSTHETIC PROPERTIES OF MAGNESIUM SALTS.

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INTRODUCTION.

IN a brief article, "The Control of Spasms by Asphyxiation,"[1] we incidentally called attention to certain resemblances in the clinical symptoms presented by animals in a state of asphyxia and by a sheep suffering from tetanus into which a large quantity of magnesium salts had been injected. Reference was made to the work of Meltzer and Auer on the anæsthetic properties of magnesium salts[2] and the injection of magnesium sulphate was based upon the amount these observers found most efficient in producing what they considered anæsthesia in rabbits.* After pointing out that the dose required to produce this phenomenon was very large (greater indeed than the therapeutic dose *per os*) and that clinical symptoms of asphyxia followed such injections, we stated that "It would seem that this factor (asphyxia) should constantly be borne in mind in interpreting the results obtained by the injection of such solutions."

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* MELTZER and AUER's views on the toxicity of magnesium sulphate are interesting. They state: "It is very little known among medical men that magnesium sulphate, which is so widely and frequently used as a purgative, is actually a very poisonous substance." American journal of pharmacy, 1906, xv, p. 387. Later in the same paper (p. 405) they state that in their experiments (rabbits, intravenous injection) "1 gm. of magnesium sulphate per kilo animal injected within one hour produced no perceptible harmful effects." The authors further state that "The absorption from the gastro-intestinal canal is perhaps so slow as not to introduce into the circulation more than the above-mentioned innocuous dose."

Applied to the average-sized man, it would follow that about 70 gm. of magnesium sulphate well diluted could be injected into the circulation within one hour without "perceptible harmful effects." In view of the above fact, and that the therapeutic dose (*per os*) is 16 gm. (U. S. Pharmacopœia, quoted by Sollmann, Pharmacology, 1908, p. 1020), it seems to us that the statement that "Magnesium sulphate is a very poisonous substance" is not justifiable.

Meltzer and Auer have taken exception to our view in a paper in which they raise the question: "Are the anæsthesia and motor paralysis caused by magnesium salts due to asphyxia?"[3] This led us to further investigation of the subject.

Frogs, rabbits, dogs, cats, rats, etc., were used, but as the results for all these animals are in agreement so far as the chief points are concerned, only a detailed account of the technique employed with frogs will be given. The results are more complete for these animals, as they lend themselves more readily to exact experimentation. Also, certain of the procedures described for frogs have not been carried out on mammals, as they were deemed unwarranted.

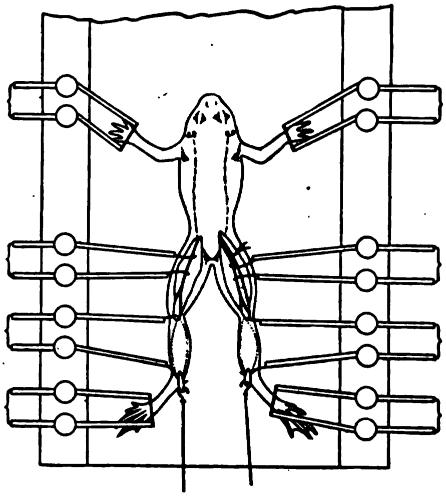

FIGURE 1. — Arrangement of electrodes on frog to study effect of subcutaneous injection of drugs. Electrodes 1, 2, 5 and 6 are shown as arranged for thermal stimulation, the thin German silver wires connecting the copper rods being heated by a galvanic current. By a suitable key, an induced or galvanic current could be conveniently led into any pair of electrodes.

I. THE ACTION OF MAGNESIUM SALTS IN RELATION TO MOTOR NERVE IMPULSES.[4]

In medium-sized frogs the femoral blood vessels in one leg (right) were ligated and the two gastrocnemius muscles connected with levers. Stationary electrodes were placed beneath the sciatic nerves, and by a suitable arrangement of keys an induced current could be led into either nerve at will (Fig. 1). After establishing the control response in both muscles, 1.0 to 1.5 c.c. of the saturated solution of the salt was introduced into the dorsal lymph sac. The nerves were stimulated at intervals and the following results recorded (Fig. 2):

1. Injection of 1.0 to 1.5 c.c. saturated solution of magnesium sulphate into the dorsal lymph sac of a medium-sized unpithed frog is soon followed by a loss of muscular response to nerve stimulation, while direct stimulation of the muscle remains effective.

The early decrease in response to direct stimulation in the non-ligated leg following the injection of magnesium salts is an exceedingly interesting phenomenon. The fact that response before injection appears to indirect stimulation with a weaker current than to direct stimulation, is probably in part due to the greater resistance presented by the muscle tissue, escape of current in surrounding tissues, etc., and hence a smaller stimulating effect than is produced by the same current applied directly to the nerves. Further, the following factors must be considered in its interpretation:

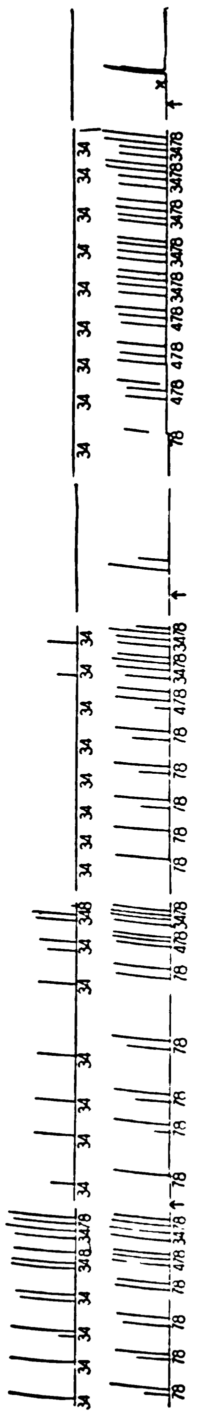

FIGURE 2. — Reduced to about one-third. April 9, 1909. Frog. 1. Weight, 45 gms. Six curves showing the effect of strong magnesium sulphate solution injected into the dorsal lymph sac of a frog. First section, direct, indirect and reflex response before, and second to sixth, after injection. Upper tracing non-ligated leg, lower, ligated leg. Beneath each are numbers referring to the point of stimulation as shown in Fig. 1. Left fore leg 1, hind leg 2, gastrocnemius muscle 3, and sciatic nerve electrode 4. Corresponding electrodes on right side 5, 6, 7, 8. At the beginning of each curve the distance between the secondary and primary coils was 12 cms., and the distance was decreased 1 cm. between each group of stimuli. The experiment lasted about an hour. The first curve shows the normal direct, indirect, and reflex response to stimulation. At the first arrow, 1.3 cc. of a saturated solution of magnesium sulphate were injected into the dorsal lymph sac. The non-ligated leg now soon ceased to respond to reflex, indirect and direct stimulation, while the responses from the ligated leg were well preserved. At the second arrow, acid was applied to the left flank. At the third arrow, the left fore leg was stimulated mechanically by pinching. At the close of the experiment fully 3 cc. of liquid were found in the dorsal lymph sac. Compared to the ligated leg, the non-ligated leg was shrunken.

A. Increased ionic concentration in the muscle, due to loss of water, and to absorption of salt. For the stimulus here is sub-maximal (cf. footnote, Fig. 3).

B. Depression of the nerve endings. For response to direct stimulation before injection probably occurs with a sub-minimal *truly* direct stimulus, the nerve endings appearing to be irritated by sub-maximal stimuli applied directly to normal muscle.

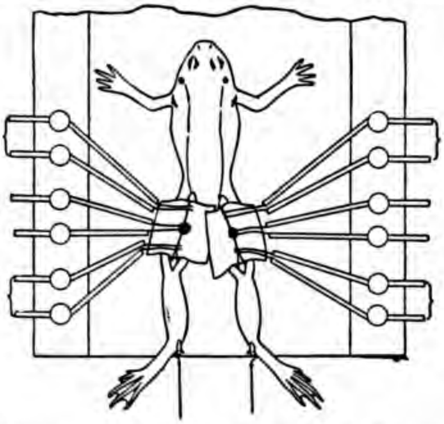

FIGURE 3. — Arrangement of electrodes to study effect of direct application of drugs to nerves.*

C. It might be assumed that since the nerves are intact, that response to direct or indirect stimulation (with the weaker currents) is due in part to a reflex factor. Then changes in the reflex centres following the administration of the drug might account for this phenomenon. But this is probably not a factor, for stimulation of the nerves of the two legs does not give similar results; and the response of the ligated leg to direct stimulation is not very similar to that of the non-ligated leg.

D. Direct injurious effect of the drug on the contractile substance itself.

2. The limb whose blood vessels were previously ligated showed no such loss of response to nerve stimulation.

3. Strong evidence was obtained that after injection of magnesium sulphate the latent period for the non-ligated leg to both direct and indirect stimulation was lengthened. These observations were of course made before complete paralysis had occurred.

The following abbreviated protocol of an experiment on a rabbit is typical of our results on mammals:

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* Without going into details, the theoretical grounding action of concentrated solutions of electrolytes when placed on the middle pair of electrodes is probably not an appreciable factor in our results. It is obvious that this factor would (theoretically at least) be greater with sub-maximal stimulating currents.

A rabbit was poisoned with magnesium salt solution administered subcutaneously. After a time one sciatic nerve was exposed and stimulated with an induced current. Upon stimulation of the sciatic nerve slight or no contraction of the muscles supplied by this nerve was observed. Direct stimulation of the muscles with a current of the same strength brought a good response. The pupils showed constriction.* At this time the animal was breathing well, and there were none of the graver symptoms of asphyxia which appeared later. The animal showed slight if any diminution of conjunctival reflexes.† Stimulation of the central end of the sciatic nerve was followed by contraction of the trunk muscles.

Thus a rabbit poisoned with magnesium sulphate shows a loss of muscular response to indirect stimulation, while at this time the muscular response to direct stimulation is good. The more peripheral skeletal muscles seem to be the first, while the muscles of respiration appear to be the last to become paralyzed.

II. THE ACTION OF MAGNESIUM AND OTHER SALTS IN RELATION TO SENSORY STIMULATION.

After ligating the femoral blood vessels of one leg in the frog, the response to sensory stimulation, *e. g.*, thermal, electrical, chemical (acid), and mechanical stimulation to the skin of the fore and hind limbs was recorded by the contraction of the gastrocnemius muscles (Fig. 1). Magnesium salts, 1.0 to 1.5 c.c. in saturated solution, were then injected into the dorsal lymph sac. After a time no response in the non-ligated leg could be elicited by stimulating as before, but the muscles in the ligated limb responded strongly. At this stage the muscles in the non-ligated leg might respond to direct stimulation, but not at all to nerve stimulation (Fig. 2). In such an experiment at this stage the animal was still breathing well and there were no indications of insensibility.*

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* We have found that pupillary changes ordinarily constitute a delicate and reliable index of asphyxia. The primary stage of *constriction*, occasionally described in text-books (STARLING, 1907, p. 404; PATON, 1905, p. 306), is quite constant. The great variation in the post mortem state of the pupil is interesting; *e. g.*, in cats it is dilated, while in rabbits it is constricted (as compared with the normal size in diffuse daylight). A fuller account of these observations will be published shortly. See Science, N. S., 1910, xxxi, pp. 395–396.

† Among surgeons the loss of the conjunctival reflex under an anæsthetic is the usually accepted sign that the patient is unconscious and insusceptible to pain. (DA COSTA: Manual of modern surgery, 1898, p. 730. Cf. PATTON: Anæsthesia and Anæsthetics, 1906, pp. 127–129.) Our own experience accords with this view.

Results.

1. The behavior of an animal in response to sensory stimulation is notably altered after the subcutaneous injection of magnesium salts in large amounts in strong solution. Motor response may even entirely disappear, but a disappearance of sensitiveness is not concomitant with motor paralysis, for if previous to the administration of the salt in frogs the blood vessels to a hind limb be ligated, the muscles of such a limb show good response to stimulation of the skin of the fore limbs.

After the injection of magnesium salts there was a stage of slight depressed reflex response in the ligated leg to stimulation of the sciatic nerve of the non-ligated leg. (At this time similar reflex response in the non-ligated leg on stimulation of the nerve of the ligated leg was only evoked with a very much stronger current.) Following this, and corresponding to the period of disappearance of muscular contraction in the non-ligated leg, the reflex response in the ligated leg to stimulation of the opposite sciatic nerve as before, was markedly heightened. This is harmonious with the asphyxial view.

Also there was evidence that at certain times reflex contraction to either cutaneous stimulation or stimulation of the opposite sciatic nerve may occur in the non-ligated leg when stimulation of its nerve with a single shock produced no effect. (This seems in agreement with the well-known fatigue experiments with the ergograph.) At this time a series of rapidly repeated electrical stimuli applied to the nerve was effective. Still later vigorous response of the ligated leg to cutting the nerve of the non-ligated leg was observed. After loss of response of the non-ligated leg to indirect stimulation, very powerful reflex and voluntary contractions were observed in the ligated leg on stimulation of the skin on the opposite side of the body either electrically, thermally, or with acid. (Voluntary is used for contractions occurring some time after application of the stimuli. In other words, the term is used advisedly for contractions coming too long after sensory stimulation to be classed as ordinary reflexes (spinal); the term cerebrospinal reflex might be cautiously employed.)

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* Cutaneous respiration is probably not an important factor in our experiments on frogs, for (1) the curare-like phenomena were marked before cessation of respiratory movements, and (2) notwithstanding cutaneous respiration the frogs soon died.

2. A rabbit poisoned with magnesium sulphate shows a loss of muscular response to indirect stimulation, while at this time the muscular response to direct stimulation is good. At this stage the reflex mechanism is still capable of functioning, as shown by the contraction of trunk muscles on stimulating the central end of the sciatic nerve. Even in a late stage, stimulation of the sciatic nerve may be followed by an increase in the rate of the respiratory movements.

3. The subcutaneous administration to wild rats of magnesium and sodium salts in large amounts in concentrated solution gives results comparable in many respects as regards production of unconsciousness (anæsthesia).

III. DIRECT APPLICATION OF MAGNESIUM AND OTHER SALT SOLUTIONS TO NERVES.

Meltzer and Auer state[6] that: 1. Solutions of magnesium salts when directly applied to nerve trunks never seem to produce irritation. 2. Such applications produce a more or less complete block for afferent and efferent, normal or artificial, impulses. 3. The more concentrated the solution the more rapid the blocking action. 4. Such a block disappears some time after the removal of the solution. The recovery is greatly accelerated by washing the nerve with Ringer's solution. 5. When applied to the sciatic nerve the sensory fibres are more rapidly affected than the motor; and when applied to the vagus the cardiac fibres are more rapidly affected than the efferent fibres for the œsophagus and the afferent respiratory and vaso-constrictor fibres.

We put certain of these statements to experimental test and also compared the action of magnesium salts with certain other of the neutral salts and with cane sugar as shown in the following experiments;

sium or other salts necessary to produce the anæsthesia phenomena of Meltzer and Auer.* We believe that any one who will attentively consider the statement[9] will see that it was employed only for the purpose of calling attention to the large dosage of the drug necessary to produce the anæsthetic effect, as this *in itself* indicates most strongly a non-specific action of the salt. Had we desired to discuss the matter from a strictly quantitative standpoint we would, of course, have taken the quantity of *plasma* rather than the blood, also the collection of liquid at the site of injection, rate of absorption into the circulating liquids, intracellular absorption, excretion of urine, etc., into account.

DISCUSSION.

Subcutaneous Injection. — Meltzer and Auer have apparently interpreted the stage of motor paralysis depicted under I and II (pp. 330–335) as showing anæsthesia.

Jolyet and Cahours[10] in 1869 stated that: In considering only the results (paralysis of voluntary and reflex movements with preservation of direct muscular excitability) which follows the introduction of the substance into the blood in dogs, and the same results with preservation of sensibility and power of movement in the preserved member (blood vessel ligated, in frogs), one cannot but compare the action of magnesium sulphate to the action of curare. Binet[11] in 1892 concluded that "Magnesium salts act upon the nervous system in a manner similar to curare. But they differ from it by the fact that the functioning of the respiratory muscles is suppressed (paralysis) more slowly." Wiki[12] in 1906 and Bardier[13] in 1907 considered this action in relation to the magnesium "anæsthesia" described by Meltzer and Auer. Our own results are in agreement with the conclusions quoted above, and seem to make further discussion on this point superfluous.

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* The production of unconsciousness through the injection of mineral acids, and the life saving action of sodium carbonate in such a condition is interesting in this connection. It seems clear that the acid phenomena, which resemble asphyxia, are due to changes associated with the decreased alkalinity of the blood. (WALTER, SCHMIEDEBERG, 1877, vii, p. 148; SOLLMANN, Pharmacology, 1908, p. 583). Meltzer and Joseph from slow intra-venous injections in dogs have concluded "that the toxicity of magnesium, calcium, potassium, and sodium to the entire animal is in inverse proportion to the amounts in which they are present in the serum of that animal." Our results from subcutaneous injections in rats of magnesium and sodium salts are in harmony with the latter conclusion. Since the grosser clinical symptoms following the administration of acids and salts in sufficient amounts are strikingly similar in many respects, an obvious query is, "Do the variations in toxicity bear a relation to alteration in the alkalinity of the blood (and other body liquids)?" It cannot be assumed *a priori* that actual determination of the total alkalinity would supply the answer to the question, for that would not necessarily be an index of the *effective* or *physiological* alkalinity. For example, although the *total* alkalinity might be unaltered, the alkalinity due to *physiological compounds* might be reduced to such an extent as to become inadequate for efficient respiratory functioning. The new (abnormal) alkaline compounds might be partially favorable but inadequate to sustain efficiently, neutral to, or directly antagonistic to such processes.

We will not at this time consider all the objections raised by Meltzer and Auer to the asphyxial factor * in "magnesium-salt-anæsthesia." Their principal argument against participation of asphyxia in the production of such an anæsthesis was that "in most of the cases *not one symptom of asphyxia was present during anæsthesia. Under no circumstance did the injection of magnesium salts ever cause an increase of the respiration in depth or frequency.*"[14] They ask[15] if *we* "ever noticed an increase in rate under the influence of magnesium salts." To this we answer yes. Furthermore, they have themselves published more than one experiment showing such an increase in the respiratory rate. One protocol shows an increase of 100 per cent following the injection of magnesium sulphate, and finally a decrease of over 50 per cent of the orignial rate (quoted below, Experiment No. 7). Illustrative of these statements are the following experiments from the paper by Meltzer and Auer just cited:

Experiment 4. *May* 4. — Gray rabbit, female, 1430 gm. (204, survivor from a previous subcutaneous injection). 76 respirations to the minute.

3.53. Injected subcutaneously into left flank 9 c.c. of a 25 per cent solution of magnesium sulphate; massaged.

4.06. Just about able to move; 128 respirations to the minute.[16]

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* The production of anæsthesia by partial asphyxiation is too well known to need discussion. (PRIESTLEY: Philosophical transactions, London, 1772, lxii, p. 147; BERT: "La pression barometrique," Paris, 1878, p. 982; GEORGE JOHNSON: LANCET, 1891, i, quoted from Text Book of Physiology, 1898, i, pp. 739–740; STARLING: Text Book of Physiology, 1907, p. 180; CUSHNY: Pharmacology, 4th ed., pp. 581–582.)

An increase of 68.4 per cent or fifty-two respirations per minute.

Experiment 7. *May* 19. — Yellow cat, male, 3820 gm.

3.40. Injected subcutaneously into left flank 15 c.c. of a 25 per cent solution of magnesium sulphate.

3.45. Respirations 64 to the minute.

4.45. Respirations 128 to the minute, deeper than before (?), salivated, staggers in cage.

4.55. Lying on the floor on one side with relaxed extended extremities; conjunctival reflex present; stepping on tail causes no reaction.

5.25. Lying on the same spot and in the same manner as before; 40 respirations to the minute, moderately deep; conjunctival reflex present; stepping on tail — no reaction; on inserting probe in nose, moves head slightly.

6.00. Respirations 28 to the minute and fairly deep; otherwise no change.[17]

The initial rate is not given, but judging from other experiments it was *probably* less than it was five minutes after injection. But taking this (64 per minute) as the initial rate, an increase of 100 per cent, or 64 respirations to the minute, and later a decrease of over 56 per cent, or 36 respirations to the minute, is shown. We have protocols (with tracings) showing similar results.

In many of Meltzer and Auer's protocols no respiratory rate is recorded. In general agreement with their published experiments in which respiratory rates are given, is their conclusion that slowing of respiration is seen in the stage of magnesium salt action, which they have termed "anæsthetic." To this we would add that preceding this, *a stage of accelerated respiratory movements* frequently occurs.

In a late stage of magnesium salt poisoning we have observed moderate quickening of the respiratory movements after strong stimulation of the central end of the sciatic nerve with an induced electrical current. Under similar conditions Meltzer and Auer have observed stoppage of respiratory movements after the application of ether to the animal's (rabbit) nose.[18] It seems, therefore, that at such a stage the respiratory centre may still be capable of responding to strong sensory stimuli. This harmonizes with the asphyxial view. For it is known that in slow asphyxiation the violent motor manifestations seen in rapid asphyxiation may be absent and the animal pass successively through the stages of stupor, unconsciousness, and collapse.[19] This does not imply that the centres are incapable of responding, but rather that the stimulating forces do not act with sufficient *rapidity* to evoke a pronounced response, *i. e.* the stimuli are subminimal. Also the *state* of the centres (and the associated responding tissues) is important in determining the result of a stimulus.

We may say, however, that we would not consider it necessary to have a primary increase in the respiratory rate in order to prove the presence of partial asphyxiation. The stage of excitation manifested by labored respirations and convulsions is not an invariable accompaniment of asphyxia. As discussed by us in a paper entitled "The Relation of Circulation to Spasms,"[20] the production or alleviation of spasms by asphyxia "depends upon the given state of metabolism (of the involved nerve cells) when the nutritional (or circulatory) change is made, and the extent of the nutritional change in the given condition." The former statement is well demonstrated by the sheep and cat protocols and the frog experiments in our paper on the "Control of Spasms by Asphyxiation."[21] When an animal is in a convulsive state with labored (maximal) respirations to begin with, we would not expect to observe manifestations of a further clearly defined stage of excitation following any method of asphyxiation. This answers Meltzer and Auer's question as to why we did not observe the symptoms of the stage of excitation in the tetanized sheep previously referred to, following the subcutaneous injection of magnesium sulphate solution. The fact that a decrease of over 50 per cent in frequency accompanied by a decrease in depth of the respirations occurred in the sheep experiment following the injection of magnesium sulphate solution is strongly indicative of a state of partial asphyxia.[*]

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[*] It is true, as MELTZER and AUER have said, we reported only one experiment (This journal, 1908, xxii, p. 442) on the injection of magnesium salts. But the clinical picture presented by this animal was so typical of the condition produced in many of their animals after similar injections, as shown by a perusal of their protocols, that we did not consider it necessary to multiply experiments. As we mentioned, the observation was made incidentally and simply included in the paper on the control of spasms by asphyxiation, since it seemed to bear a certain elation to that subject.

In the experiment briefly described on page 333, the animal showed a good conjunctival reflex (touch) until a decrease of over 80 per cent in the rate of respiratory movements had occurred. As above indicated, a number of Meltzer and Auer's protocols indicate a marked decrease in the respiratory movements before pronounced symptoms of anæsthesia were observed. It would seem, therefore, that ***pulmonary ventilation*** bears a very constant relation to the anæsthesia phenomena under discussion.

Meltzer and Auer's statements regarding cyanosis are open to criticism. Magnesium salts produce a decrease in the pulmonary ventilation, and we have repeatedly observed cyanosis following their injection.

Finally, subcutaneous injections of other salts in large amounts are followed by "anæsthesia" phenomena comparable in many respects to those following the injection of magnesium salts, the differences appearing to be chiefly of a quantitative nature.

We must add that in recent writings Dr. Meltzer appears to be taking a more conservative view as to the interpretation of the phenomena following the injection of magnesium salts. For example, he states that "A noteworthy point in the fatal terminations of the dogs from infusion (intravenous) of sodium chloride is the fact noted by us that in all experiments the respiration subsided several minutes before the heart stopped and without any terminal convulsions. From the observations of Locke, Cushing, and others we know now that by perfusion of the posterior extremities with sodium chloride the indirect irritability disappears long before the direct; in other words, sodium chloride exerts upon the motor nerve endings a 'curare-like' action. May not the early stoppage of respiration in these experiments without accompanying convulsions have been due to such a 'curare-like' action of the sodium chloride upon the motor nerve endings?[22] . . . Magnesium exerts also a depressing effect upon muscle and motor nerve endings or, as it is frequently expressed, upon direct and indirect muscle irritability. . . . The depressing action upon the motor nerve ending magnesium shares with most of the other inorganic ions of the animal body. It is the 'curare-like' action which is exerted by sodium chloride, potassium chloride. . . . The curare-like action of magnesium upon the peripheral motor nerve endings has surely some share in the danger which comes from the respiratory paralysis. With an increasing depression of the irritability of the nerve endings of the respiratory muscles, be it ever so small, is a grave factor."[23]

Direct Application to Nerves. A. *Stimulating Action.* — Meltzer and Auer's position[24] is indicated by the general statement that, "As we have pointed out in the previous papers, we have so far noticed not a single phenomenon among the effects of these salts which could be reasonably interpreted as an irritating influence." They used rabbits. Our own results show that magnesium salts in common with numerous other crystalloids exert a very decided stimulating action when applied directly to the exposed trunk of a sciatic nerve of an otherwise intact frog.* Indeed magnesium chloride stimulated more powerfully than certain other of the substances. Such action may affect afferent fibres, as evidenced by powerful reflex contractions. As the reflex factor was present in all our experiments, we cannot make a *positive* statement regarding a purely efferent effect, but from the similarity of action with other salts which are known to possess such action, *e. g.* sodium chloride, a reasonable doubt as to such action is barely possible.

B. *Conductivity.* — As to the explanation of the difference in the time of blocking of different fibres by the direct application of salt solutions, we believe Meltzer and Auer's interpretation[25] attributing it to a difference in irritability in the different fibres is more rational than the explanation attributing it to a difference in the irritability of the nerve endings.[26]

Better still we believe is the hypothesis advanced by Dixon in explaining the difference in time of blocking to different kinds of fibres after the direct application of cocaine,[27] namely: 1. Such a distribution of the fibres within the nerve (vagus) as to expose one kind more than the other to the action of the drug; 2. That one kind of fibres has a greater chemical affinity than the other for the drug (cocaine).

To the above considerations we would add *a difference in susceptibility to the action of the drug* whether the action be exerted through chemical, physical, physico-chemical means or otherwise, *e. g.* vasoconstriction. For it is well known that tissues differ in their susceptibility to anæmia (asphyxia), and that different elements of the same tissue exhibit a similar difference in susceptibility. This is strikingly evident in the central nervous system.[28]

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* **Brainless frogs gave essentially similar results.**

Illustrative of such differences in peripheral nerve fibres may be mentioned the persistence of pronounced vasodilator response to stimulation of mixed nerves after cooling, etc., at a time when vaso-constrictor effect has become much reduced.[29] So that the view that the difference in time of disappearance of conductivity after the application of agents is due to the difference in resistance (or susceptibility) to the adverse condition is rational. We believe that to attribute the ultimate action of at least a large proportion of such agents to *asphyxiation* is in accord with observations hitherto recorded. The sole consideration impelling us to assert our allegiance to this view as a factor under the above conditions is that it rests upon a perfectly definite phenomenon, *the relative susceptibility of tissues to asphyxia or associated nutritional disturbance.*

In the case of concentrated salt solutions, such as we have employed, in line with this view, is the abstraction of water from the tissues, so the action would be in part at least physical. In the case of iso- and hypotonic solutions, diffusibility of the substance would have to be taken into account as well as any other property it might possess whereby the internal respiratory or associated processes of the tissues subjected to its action might be influenced.

Although we have not studied the action of dilute (iso- and hypotonic) solutions of magnesium salts on the conductivity of nerves, we do not believe they can thus play any important part in the general loss of sensibility observed after the subcutaneous administration of large amounts of such salts in strong solution, for: 1. A block in a nerve produced by strong sodium chloride solution may be removed by washing with isotonic solution of the same salt; 2. Study of such blocks induced by the application of hypertonic solutions indicates complete afferent before complete efferent block. But when administered subcutaneously, motor response disappears before sensory. That loss of muscular response is not due to loss of motor conductivity is clearly shown by our experiments. For example, in a ligated limb the persistence of muscular response to sensory stimulation at a time when muscular response to even strong stimulation of the efferent nerves evoked no response in the non-ligated limb.. Finally, Meltzer and Auer's results from the direct application of dilute solutions, namely, that "the local anæsthetizing effect of the solutions has been, as a rule, in proportion to their concentration,"[30] support this view.

C. *Excitability.* — The effect of direct application of magnesium salts to nerves on excitability has not been so closely observed. Meltzer and Auer briefly present the results they obtained on stimulation of the nerve at the point where the magnesium salt solution was applied.[31]

Some evidence is adduced to indicate that irritability was more profoundly affected (reduced) than conductivity but they state that the results are inconclusive.[32] We can add nothing to this at the present time, as our experiments with solutions made of comparable concentrations (taking the isotonic co-efficients[33] of the substances into account) are incomplete.

CONCLUSIONS.

We conclude, therefore, that a very marked early effect of magnesium salts when injected subcutaneously in sufficient doses is a paralysis of the peripheral neuro-muscular apparatus of voluntary muscles. An animal in such a condition is sensitive, but ordinarily does not respond to sensory stimulation because it is paralyzed. Paralysis of the more distal skeletal muscles is followed by a gradual paralysis of the respiratory muscles which results in a decrease in the pulmonary ventilation. This, in conjunction with the probable action of the salt upon other processes concerned in respiration[*] produces a state of partial asphyxia manifested by the usual symptoms. Anæsthesia may or may not occur at this stage, depending upon the degree of asphyxiation.

Briefly, our further investigations corroborate our previous position. Apparently the anæsthesia that may follow the injection of magnesium sulphate solutions is due chiefly to asphyxia. ***Magnesium salts cannot be regarded as having marked specific anæsthetic properties.***

[*] See pp. 337, 338.

Bibliography.

[1] **Ryan** and **Guthrie**: This journal, 1908, xxii, pp. 440–444.
[2] **Meltzer** and **Auer**: *Ibid.*, 1905, xiv, p. 366; 1906, xv, p. 387.
[3] **Meltzer** and **Auer**: *Ibid.*, 1908, xxiii, p. 141.
[4] **Guthrie** and **Ryan**: Society for Experimental Biology and Medicine, 1910, vii, p. 39.
[5] **Meltzer** and **Auer**: This journal, 1906, xvi, p. 250.
[6] **Guthrie**: Archives of internal medicine, 1910, v, p. 244.
[7] **Meltzer** and **Auer**: This journal, 1908, xxiii, p. 143.
[8] **Ryan** and **Guthrie**: This journal, 1908, xxii, p. 443.
[9] **Ryan** and **Guthrie**: *Ibid.*
[10] **Joylet** and **Cahours**: Archives de physiologie, 1869, pp. 113–120.
[11] **Binet**: Revue médicale de la Suisse romande, 1892, pp. 523, 593.
[12] **Wiki**: Journal de physiologie et pathologie générale, 1906, pp. 793–803.
[13] **Bardier**: *Ibid.*, 1907, ix, p. 611.
[14] **Meltzer** and **Auer**: This journal, 1908, xxiii, p. 145.
[15] **Meltzer** and **Auer**: *Ibid.*
[16] **Meltzer** and **Auer**: *Ibid.*, 1905, xiv, p. 370.
[17] **Meltzer** and **Auer**: *Ibid.*, p. 376.
[18] **Meltzer** and **Auer**: *Ibid.*, p. 373.
[19] See **Sollmann**: Pharmacology, 1908, p. 458.
[20] **Ryan** and **Guthrie**: Quarterly Bulletin, Medical Department, Washington University, 1908, vii, p. 58.
[21] **Ryan** and **Guthrie**: This journal, 1908, xxii, p. 440.
[22] **Joseph** and **Meltzer**: Journal of pharmacology and experimental therapeutics, 1909, i, p. 20.
[23] **Joseph** and **Meltzer**: *Ibid.*, pp. 384–385.
[24] **Meltzer** and **Auer**: This journal, 1906, xvi, p. 234.
[25] **Meltzer** and **Auer**: *Ibid.*, p. 247.
[26] **Meltzer** and **Auer**: *Ibid.*, p. 251.
[27] **Dixon**: Journal of physiology, 1905, xxxii, p. 87, quoted by **Meltzer** and **Auer**, This journal, 1906, xvi, p. 243.
[28] **Stewart**, **Guthrie** and **Pike**: Journal of experimental medicine, 1906, viii, pp. 289–321.
[29] **Porter**: American text book of physiology, 1903, i, p. 197.
[30] **Meltzer** and **Auer**: This journal, 1906, xvi, p. 238.
[31] **Meltzer** and **Auer**: *Ibid.*, pp. 238–239.
[32] **Meltzer** and **Auer**: *Ibid.*, p. 239.
[33] **De Vries**: Zeitschrift für physiologische Chemie, 1888, ii, p. 427. See **Jones**: Elements of physical chemistry, 1909, pp. 252–253.

THE RELATION OF THE PANCREAS TO THE BLOOD DIASTASES IN THE DOG.

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THOUGH numerous investigators have concerned themselves with the diastases of the blood, little information has been brought forward which would indicate the source or seat of their production in the animal body. This work was undertaken at the suggestion and under the direction of Dr. Carlson, to determine if possible, the rôle of the pancreas in their formation. While a number of men have investigated the diastases after removal of the pancreas with partly conflicting results, no one seems to have followed the progressive changes in concentration in the blood. We expected that if the pancreas were responsible for their formation or destruction, the amount of diastases in the blood would show a gradual fall, varying with the rapidity of elimination or destruction of the ferments; while if the pancreas played no direct part, there should be little or no change. It was found that the problem is not so simple, but is complicated by other factors to be discussed in connection with our experiments.

LITERATURE.

While Bainbridge and Beddard[1] found in two diabetic patients an almost complete lack of blood diastases, the serum of other diabetics showed no change in concentration. They found that after pancreatectomy in one cat, there was no disappearance of the blood diastases.

Carlson and Luckhardt[2] also record that the removal of pancreas from cats did not greatly affect the concentration of blood and lymph diastases.

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[1] Bainbridge and Beddard: Biochemical journal, 1907, ii, p. 89.

[2] Carlson and Luckhardt: This journal, 1908, xxii, p. 148.

Schlesinger[3] states that after pancreatectomy the blood diastases disappear completely. The basis of his claims appears to be the results which he says confirm the work of Lépine and von Kaufmann, that the blood diastases were reduced to one-third, twenty-four hours after removal of pancreas. No further observations were recorded on these same animals. Schlesinger also found that in a dog who had had the greater part of the pancreas removed for several months, the diastases were decreased considerably. Since this observer found that ligating the duct of Wirsung increased the concentration of diastases in the blood, he concludes that the blood diastases are simply amylopsin absorbed from intestines or pancreas. Carlson and Luckhardt, however, found that the goat and the sheep have practically no diastases in the blood, though these animals on account of their diet obviously secrete abundant amylopsin into the gut.

Borchardt[4] holds that the properties of the blood, lymph and liver diastases are identical and considers the blood diastases to be simply the liver diastases passed into the blood and lymph.

Haberlandt[5] concludes that the blood diastases come, at least in part, from the leucocytes, since in leucocytosis, in warm blooded as well as cold blooded animals, the body fluids show increased diastatic action. He also claims that the serum diastases are increased the longer the serum stands in contact with the clot, and consequent progressive destruction of the leucocytes.

After perfusion sufficient to remove the blood and lymph, Macleod and Pearce[6] found that by suitable methods of breaking up the liver cells, an additional amount of a glycogenolytic ferment is secured, and conclude that this ferment exists as an endoenzyme. But they leave unsettled whether it is produced by the liver cells, or brought to the liver in the blood and fixed by the liver cells.

METHODS.

Dogs were used in all experiments. The pancreas was removed by blunt dissection under ether anaesthesia, care being taken to leave intact the duodenal circulation.

[3] SCHLESINGER: Deutsche medicinische wochenschrift, 1908, No. 14, p. 593.

[4] BORCHARDT: Archiv für die gesammte Physiologie, 1903, c, p. 259.

[5] HABERLANDT: Archiv für die gesammte Physiologie, 1910, cxxxiii, p. 175.

[6] MACLEOD and PEARCE: This journal, 1910, xxv, p. 255.

A 10 c.c. sample of blood was drawn just before the operation, and samples taken the succeeding day, and at intervals of about three days thereafter until the animal died. In the case of the pancreatectomized dogs the blood was drawn from a cut in the ear; in other cases from a leg vein through a hypodermic needle.

For the determination of the concentration of the diastases the method used was that found satisfactory by Salkowski,[7] Macleod and Pearce, Schlesinger, and previously used in this laboratory by Carlson and Luckhardt. The blood was defibrinated by stirring, centrifugated, serum drawn off and placed in ice-box till used.

A one per cent solution of arrow-root starch was prepared just before using by boiling five minutes. In the first experiment ½ c.c. of serum was added to 25 c.c. of this starch solution and incubated at 38° C., and tested at one-half hour intervals with a very dilute solution of I in KI, for the complete disappearance of the iodine blue reaction. As the end point was approached, tests were made every fifteen minutes. In the other experiments 20 c.c. of starch solution and 1 c.c. of serum were used.

The serum was added practically simultaneously to the starch in uniform flasks, at the temperature of the thermostat, and shaken with the same intensity for the same length of time. Duplicates of two to three samples were used in most instances, and were found to agree in all cases, indicating that the method was without material error.

RESULTS.

From the table and curves given it will be seen that, on the day following pancreatectomy, the diastases are very markedly decreased in all cases. In one case, (Dog *A*) on the fourth day after the operation, there was a slightly lower concentration of blood diastases than the first day after the operation, with a slight return toward normal on the seventh day, and on the tenth day reaching a level which was held until the death of the dog on the nineteenth day after the operation. Dog *B* showed the least diastases on the day after the operation, returning by the seventh day to a constant level considerably below the normal, which was maintained till the dog was killed on the seventeenth day. Dog *J* behaved similarly, although the diastases fluctuated within narrow limits. In all these cases autopsy showed that all pancreas tissue had been removed. The dogs showed the usual symptoms of pancreatic diabetes; hunger, thirst, polyurea, characteristic fatty feces, and gradual wasting. Fractional coagulation of the blood was noted a number of times in dogs *B* and *J*, indicating diminished concentration of the prothrombin.

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[7] SALKOWSKI: Archiv für pathologische anatomie (Virchow), 1888, cxx, p. 343.

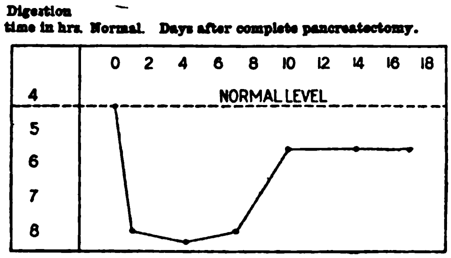

FIGURE 1. — Dog A, using 25 c.c. of 1 per cent starch solution + ½ c.c. of serum.

On four other dogs pancreatectomy was performed, but all died one or two days after the operation. Dog *D* showed at autopsy a small focus of infection on the ileum; otherwise normal. Dog *C* had no peritoneal infection and no reason was apparent for its death. Dog *E* died of intussusception. In these four cases there was the same sharp and marked fall in the concentration of the diastases in the twenty-four hours following the operation.

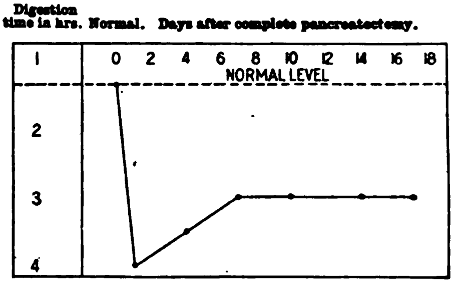

FIGURE 2. — Dog B, using 20 c.c. of 1 per cent starch solution + 1 c.c. of serum.

In order to determine if the above results are in part or wholly due to such factors as anaesthesia, trauma and shock, the following experiments were undertaken as controls. Dog *F* was kept under anaesthesia for the same length of time as dog *E*. A similar abdominal incision was made as for pancreatectomy, traction made on intestines, duodenum exposed and all accessory conditions of pancreatectomy fulfilled. It was found that there was a slight drop from one and one-third to two hours for diastatic action to be completed. On the third day the concentration was nearly back to normal, fluctuating later between two and one-half hours. All the variations in this case seem to be within the range of normal variations of the concentrations of the diastases of the blood.

In another series the effect of ether anaesthesia was studied. In

Dog *H*, anaesthetized for three hours, there was a fall in diastases from one and one-half to two and one-sixth hours digestion time, returning to normal on the third day. In three other dogs anaesthesia of two and one-third, one and three-fourths and one and one-half hours respectively, failed to produce any marked effect, the diastatic power of the blood being tested, just before and just after anaesthesia, and twenty-four hours later.

TABLE I.

RATE OF DIGESTION OF STARCH SOLUTION BY DOG'S SERUM AFTER COMPLETE PANCREATECTOMY. IN EXPERIMENT A, 25 C.C. OF 1 PER CENT STARCH SOLUTION +½ C.C. OF SERUM WAS USED. IN OTHER EXPERIMENTS 20 C.C. OF 1 PER CENT STARCH SOLUTION +1 C.C. OF SERUM.

Dog.	Digestion time before operation in hours.	Day on which observation was made, and digestion time after operation in hours.												
		1	3	4	6	7	9	10	12	14	15	17	19	21
A	4¼	8	..	8¼	..	8	..	5½	..	5½	..	5½		
B	1¾	4	..	3½	..	3	..	3	..	3	..	3		
J	1¾	4 1/12	4 1/12	..	4½	..	4½	..	3½	..	4	..	3⅙	3 7/12
C[1]	1¾	2½												
D[1]	2¼	2¾												
E[2]	2	4½	8											
I[1]	1½	4½												

[1] Dog died third day after operation. [2] Dog died fourth day after operation.

SUMMARY AND DISCUSSION.

A study of the curve of diastatic concentration in the blood after pancreatectomy indicates that the diastases in the blood are either destroyed or eliminated in a short time, as the first rapid fall comes on within twenty-four hours. Their rapid disappearance speaks for a constant production in the body, since Carlson and Luckhardt showed that in individuals of the same genus the concentration of diastases varies normally only within narrow limits.

These experiments also show conclusively that diastases are not formed solely in the pancreas. Otherwise the curve of their concentration would show an approach to zero, instead of showing a slight increase toward the end.

That the pancreas plays an important rôle in the production of the diastases or in their destruction, either directly or indirectly, is indicated by the following facts. The diminished concentration of the diastases cannot be explained by the general condition of the animal, as in our experiments the failure of the diastases does not keep pace with the progressive marasmus. Wohlgemuth,[8] Carlson and Luckhardt, Macleod and Pearce, found that the amount of diastatic ferments of the blood was independent of the state of nutrition or kind of food. Therefore, we conclude that since diet and condition of animal does not necessarily influence the concentration of the diastases, and since, after pancreatectomy, this concentration approaches a constant level, neither falling below a certain point, nor returning to normal, the difference in diastases must be due then to a loss of specific diastase producing function or altered rate of destruction, due directly or indirectly to removal of the pancreas. It does not seem that the greater amount of diastases in the blood with the pancreas intact could be due to the resorption of amylopsin poured into the intestines, since the goat and the sheep, as found by Carlson and Luckhardt, have practically no diastases in the blood, though the nature of their diet would necessarily require a considerable amount of that ferment in the pancreatic juice.

Since the concentration of the diastases in the blood and in the lymph does not seem to parallel the hyperglycemia and diabetes after pancreatectomy, the diastatic ferments, as Schlesinger has pointed out, seem to have no direct relation to sugar metabolism, and as Carlson and Luckhardt suggest, appear to be waste products. It may be, however, as Schlesinger considers, that in some mammals the main bulk of the blood diastases are pancreas diastases reabsorbed from the external secretion. If this is so, then on removal of the pancreatic factor, the blood and lymph diastases remaining would be those of the liver and other tissues, such as the intestinal mucosa, and possibly the leucocytes.

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[8] Wohlgemuth, J.: Biochemische Zeitschrift, 1909, xxi, p. 381.

Two factors must then be considered in connection with the diastases of the blood. First, the diastases of pancreatic origin, exogenous in a sense, and of no significance in carbohydrate metabolism; and second, endo-enzymes, possibly largely from the liver, which before leaving the tissue cells may play an important rôle in the destruction of glycogen.

The explanation of the gradual approach to normal ferment concentration after the initial diminution following the removal of the pancreas in dogs is not obvious. Further light must be sought by similar studies on different mammals, as the work of Bainbridge and of Carlson and Luckhardt seems to show that cats exhibit much less diastase diminution than dogs after pancreatectomy. If the approach towards the normal diastase concentration is an attempt of the organism to re-establish the original equilibrium in the body fluids, it would seem that in the dog at least the blood and lymph diastases play some rôle in metabolism.

We wish to express our appreciation for the constant help and encouragement of Dr. A. J. Carlson during the progress of this work.

THE EFFECTS OF THYROIDECTOMY ON THE RESISTANCE OF RATS TO MORPHINE POISONING.

By W. H. OLDS, Jr.

[From the Hull Physiological Laboratory of the University of Chicago.]

IN order to test the commonly accepted view that the thyroid secretion reaches the blood by way of the lymph, *i. e.*, through the thyroid and neck lymphatics, Carlson and Woelfel applied several tests, or supposed tests, of these secretions to the lymph collected directly from the thyroid glands. The different tests yielded negative results. This was true even of the Hunt acetonitril test, although Hunt estimates that this test is forty times more delicate than the test for iodine. Both Hunt and Carlson have raised the question whether this is a test for thyroid secretion, or is due to some other constituent, such as the iodine itself.

The work here reported was undertaken at the suggestion of Dr. Carlson, for the purpose of determining whether the results obtained by Hunt were due to the thyroid secretions. If the diminished resistance of the rat to morphine is due, as Hunt believes, to the additional amount of thyroid secretion in the body of the animal, a diminution of the secretion by thyroidectomy ought to cause an increased resistance to morphine poisoning. Or, again, if the decreased resistance is due to the action of the thyroid secretion upon the other organs or tissues of the body, diminution of the secretion should raise the resistance. If this action is such a delicate test for thyroid secretion as is advocated, even a slight diminution of the secretion should raise appreciably the resistance to morphine.

LITERATURE.

The literature contains little information bearing directly upon the question, except, of course, the papers of Hunt, and Hunt and

Seidell.[1] Hunt used white mice to a great extent in his work and poisoned them with acetonitril. The effects in these cases he found were exactly opposite in rats to what they were in mice. Morphine, however, acted alike in both genera.

Thyroid was fed in varying amounts to the mice. Acetonitril was then injected subcutaneously. Hunt's results show that the mice resisted a dose of acetonitril three or four times as large as the dose fatal to normal mice. Feeding 0.005 gm. of sheep thyroid per day caused the mice to resist about eight times as much acetonitril as the controls. Carlson and Woelfel have partly confirmed these results.[2] Thyroids that contain no iodine, or rather that give no test for iodine, will cause an increased resistance; yet Hunt's results show that the difference in resistance to poisoning varies almost exactly as does the iodine content of the thyroids fed.

Hunt applied this test for thyroid with blood taken from patients suffering with exophthalmic goitre. He fed the blood to mice and poisoned them subsequently with acetonitril as in the other experiments. In the first case the mice resisted 0.15 mg. of acetonitril per gram weight more than did the controls. In another case the results were not conclusive, while in a third they were negative. Carlson and Woelfel made this same test with blood taken from a typical case of exophthalmic goitre but obtained negative results.[3]

Hunt reports that several organs of the body possess a substance which has a similar physiological action. When fed to mice they render them less susceptible to acetonitril poisoning. He adds, however, that these organs are less active, possibly because of a lower iodine content. He also states that inorganic iodine given in the food increases the resistance of white mice to acetonitril poisoning, but much less than thyroid itself.

Besides these experiments upon mice, Hunt records a number of experiments upon white rats. His results show that after thyroid feeding the rats are less resistant than before to morphine poisoning. From his reports it seems that feeding thyroids to rats may lower their resistance as much as ten times. The controls would survive a dose of morphine ten times as large as that necessary to kill the thyroid fed rats. Here also, as in the mice experiments, the physiological activity varied almost exactly as did the iodine content.

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[1] HUNT and SEIDELL: Hygienic Laboratory, 1909. Bulletin No. 47, Marine Hospital Service of the United States.

[2] HUNT: Journal of biological chemistry, 1905, i, p. 1; Journal of the American Medical Association, 1907, xlix, p. 240.

[3] CARLSON and WOELFEL: This journal, 1910, xxvi, p. 32.

EXPERIMENTAL METHODS.

In our experiments white rats were used throughout. The same work is now being done in our laboratories upon white mice by Mr. Lussky. In our present experiments the rats were all in first class condition, being well fed and physically active. They were thyroidectomized and allowed to recover from the operation. The operation is comparatively simple. As is well known, the thyroids in the rat are relatively large organs lying directly upon the trachea. The blood supply is abundant. The superior thyroid artery comes into the gland at the upper pole. The inferior thyroid artery is variable, but usually enters the gland a few millimetres from the inferior pole. The recurrent laryngeal nerve runs close to the gland, between it and the trachea, and is very closely associated with the inferior thyroid vein. If the recurrent laryngeal nerves are injured, the animal develops a peculiar choky respiration which sounds as though there were some obstruction in the trachea. These symptoms may pass off in from one to four days but frequently end in death. But when the proper precautions are taken the operation is very successful. The animal is up and eating in from five minutes to an hour, depending upon the depth of the anaesthesia. In only one case were other symptoms noticed. In this case Dr. Carlson noticed typical tetanic spasms seven days after operation. This rat died the same afternoon. The cause of these spasms was probably due to removal or extensive injury to the para-thyroid glands. Other rats that died may have had similar attacks without being noticed; for no attempt was made to keep them under special observation. In the majority of cases, however, it was impossible to tell the operated rats from the normal ones excepting by the scar. This period of recovery extended from eight to twenty-eight days. The rats were then weighed and injected with morphine, so many milligrams per gram weight of rat, and the minimum fatal dose determined as nearly as possible. Control rats were taken in all cases from the same lots as the thyroidectomized rats of the same series. The fatal dose for the controls having been determined, a slightly larger dose was injected into the thyroidectomized rats. In this way there was no waste of thyroidectomized rats. When non-fatal doses are given the rats cannot be used again, as they develop some tolerance. The increased dose was so slight that had there been any increased resistance this dose of poison would have been readily overcome. In only one case did the thyroidectomized rat overcome the morphine, but the increased dose was so slight that this one result can be readily explained upon the ground of individual variation.

It might be argued that the thyroids were not completely removed. All that can be said is that all visible thyroids were removed. Postmortem in all cases save one showed that the glands were entirely removed. In the exceptional case a small piece of tissue was found, at the site of operation, that might have been thyroid tissue. Microscopical examination disclosed no typical thyroid tissue, however. But even if the thyroids were not completely removed, certainly the greater part of thyroid tissue was eliminated and consequently there must have been a great decrease in thyroid secretion. There is a possibility that the secretion might be fixed in the body tissues or held in the blood and other fluids, but during a period of twenty-eight days this secretion would in all probability be greatly reduced in quantity, so that the supposed test would still hold good.

RESULTS.

The results on the thyroidectomized rats and upon the normal controls are given in Tables I and II. It will be seen that the fatal dose is practically the same for both series, 0.33 to 0.34 mgs. per gram body weight. It is therefore obvious that the removal of the thyroids and the consequent diminution of the concentration of the thyroid secretion in the body has not appreciably altered the resistance of the rats to morphine.

If all the thyroid tissue was not removed, certainly the greater part of it was, and the thyroid secretion therefore materially diminished. Since the rate of destruction of the secretion is not known, we can only assume again that it must be diminished, at least, to some extent twenty-eight days after the thyroids are removed. There

TABLE I.

Resistance of Thyroidectomized Rats to Morphine Poisoning.

No. of days between operation and injection.	Weight of rat	Morphine per gram weight of rat.	Results.
	gm.	mg.	
14+	138	0.42	Died
14	102	0.39	Lived
"	170	0.52	Died
"	158	0.40	"
"	203	0.35	"
"	118	0.46	"
17	139	0.40	"
15	113	0.35	"
24	132	0.40	"
12	136	0.35	"
28	166	0.35	"
14	92	0.35	"
"	154	0.35	"
9	167	0.35	"
18	182	0.34	"
18	142	0.34	"
18	92	0.34	"
22	139	0.34	"
27	189	0.35	"
27	128	0.34	"
20	184	0.35	"
27	175	0.34	"
22	160	0.34	"

may be compensation by other organs of the body after the thyroids are removed, but in this case the thyroid secretion, which is the basis of the test, has given way to another substance or substances. This substance coming from other organs certainly cannot be thyroid secretion. If it answers to the Hunt test, then the test is not one for thyroid secretion specifically. From this it follows that the Hunt test cannot be used as a test for the concentration of the thyroid secretion in the blood and lymph.

TABLE II.

Resistance of Normal Rats to Morphine Poisoning.

Weight of rat.	Morphine per gram weight of rat.	Results.	Weight of rat.	Morphine per gram weight of rat.	Results.
gm.	gm.		gm.	gm.	
132	0.32	Lived	98	0.32	Lived
174	0.42	Died	114	0.32	"
96	0.40	"	117	0.32	"
106	0.40	"	162	0.32	"
104	0.35	"	148	0.31	"
109	0.43	"	64 [1]	0.34	"
97	0.43	"	56 [1]	0.34	"
99	0.43	"	55 [1]	0.35	"
99	0.45	"	58 [1]	0.35	"
121	0.30	Lived	52 [1]	0.36	Died
92	0.43	Died	..	...	..

[1] These last five experiments were made upon young rats all taken from the same litter (about ten weeks old). They were used simply to determine how much variation there might be in young rats. They seem to show that young rats are slightly more resistant to morphine poisoning than old ones.

Conclusions.

1. Rats will survive without harmful results after thyroidectomy, provided the recurrent laryngeal nerves are not injured, and the parathyroid glands not entirely removed.

2. Thyroidectomized rats show the same resistance to morphine poisoning as normal rats, at least, within a period of eight to twenty-eight days after the operation. These facts seem to question or at least limit the Hunt test as a test for the concentration of thyroid secretion in the body fluids.

THE MECHANISM OF TONE IN PLAIN MUSCLE.

By D. J. LINGLE.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

NERVE and muscle tissue exhibit a form of activity which, from its special character, has received the name of tone. This tonic activity in muscle seems, in some respects, to shade gradually into what may be called its grosser physiological functions, but in other ways it manifests certain distinct differences. Probably the best definition of tone, and the one harmonizing closest with the ideas of a majority of physiologists, would be: "Tone is a state of partial continuous activity in the elements of a tissue." [1] It is assumed that this state of slight or partial activity varies from time to time, and may be increased or decreased from a certain mean.

Such a conception is peculiar, and, in accepting it, we are compelled to adopt ideas that are, to say the least, not in harmony with some fundamental physiological facts. The definition postulates a form of activity that is continuous and seemingly never manifests fatigue. For example, the elements in the wall of a blood vessel are in a state of tone during life. They never entirely cease to act and are never fatigued. How is this to be reconciled with the fact that plain muscle, excised and artificially stimulated, fatigues with ease? [2] Biedermann suggests that in the case of tone we are dealing with extremely feeble physiological processes where the necessary chemical changes are of so low an order that katabolism and anabolism balance, and fatigue is accordingly absent. In other words tone is the result of peculiar and, we may say, a unique form of physiological activity.[3] Bottazzi has endeavored to show that in heart muscle, tone activity and contraction activity are functions of distinct structures.[4] But granting this, his conception implies that in tone the sarcoplasmic elements are in a state of continuous activity which simply transfers the difficulty from one to another structure. The fundamental defect in all conceptions of tone consists in this, that they violate or ignore the universal law that work produces fatigue, and necessitates rest for repair. And further, they assert that muscle manifests two more or less distinct forms of activity. One, the ordinary contraction, the other, the form that produces tone.

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[1] SHERRINGTON: SCHÄFER'S Text-book of physiology, 1900, ii, p. 870.

[2] COLIN STEWART: This journal, 1901, iv, p. 205.

[3] BIEDERMANN: Archiv für die gesammte Physiologie, 1904, cii, p. 503.

[4] BOTTAZZI: This Journal of physiology, 1897, xxi, p. 1.

The object of the present paper is to present a possible way around this difficulty. It will attempt to show that tone in plain muscle may be the result of normal and usual modes of activity; that the structures involved work, are fatigued, and are restored by periods of rest like all other tissues, but that tone itself does not show fatigue because of the peculiar way the apparatus is used. This idea has been gained by the histological study of tissues in a state of tone in the sinus muscle of the turtle's heart, in the plain muscle of the intestine and stomach, and in the sphincter muscle of the stomach of the frog and pecturus. The conception of this mechanism of tone is based upon an interpretation of what is seen where structures, in various stages of natural and artificially produced tone, are examined with the microscope. And in these examinations it is assumed we can determine the state of activity of plain muscle cells by the shape of their nuclei.[5] The nuclei are slender and elongated in resting cells, and in fully contracted cells are short and oval in outline.[6] The correctness of this assumption is supported by so many writers that it seems justifiable to use it as a basis in interpreting what is seen in tissues in various conditions of tone.

If tissue, like the sphincters, is examined when in strong tone, it will be seen that many of the nuclei are in the condition characteristic of fully contracted cells. But not all. Scattered about, and mingled with them, are other nuclei that are characteristic of resting cells, and still others that are apparently in states of activity intermediate between the two extremes. It is not easy to get relaxed sphincters, but, in specimens approaching nearest this state, nuclei in all three stages can be found. Some indicate cells in as strong state of contraction as are the cells of tissue in strong tone. These are less numerous, however, and the number of inactive cells is greater. Nuclei indicating intermediate stages of activity are also present. A similar state is seen in strips of plain muscle artificially stimulated, and in contractions produced normally. Even when the strip's contraction is maximal it always contains many resting cells mingled with active ones, and also a proportion of intermediate stages. It seems, then, that when plain muscle works normally we never find all the cells active at the same time.

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[5] HENNEBERG: Anatomische Hefte, 1901, xvii, p. 425; FORSTER: Anatomische Anzeiger, 1904, xxv, p. 338; McGILL: The American Journal of Anatomy, 1909, ix, No. 4, p. 494.

[6] FORSTER lays considerable stress on the fact that he found the nucleus in activity spirally twisted. In my preparations made as described on pages 6 and 7, this condition is rarely found. I believe this appearance is a result of his technique, and not physiological.

A similar condition seems to be characteristic of sinus tissue from the turtle's heart. Here we have to judge of the state of activity by the position of the muscle bands and the diameter of the fibrillae. In strong tone part of the fibrillae are active; others are resting. And in feeble tone fibrillae can be seen contracted as fully as any found in tissue in a more active condition.

Henneberg and Forster, *loc. cit.*, in their papers on plain muscle of the arterial wall, both call attention to the fact that in all conditions of the wall there is a certain percentage of cells in the inactive state.

These conditions, observed in the sinus of the heart, blood vessels, wall and plain muscle when in artificial and natural tone, have suggested an idea of tone mechanism somewhat different from the one involved in Sherrington's definition given at the beginning of this paper. It may be that tone is not the outcome of a state of partial simultaneous activity of all the elements of a tissue; but is rather due to the interlacing activity of only a part of the elements at any one time; that the individual elements concerned in producing tone as a result of this activity become fatigued, and can then cease to work, and rest. While these elements are resting another set is active in their place. In this way the labor of maintaining tone is constantly shifting from tired to rested elements. The nuclei of various stages in tissue in strong tone belong, some to fully contracted cells, others to cells that have been producing tone and are relaxing, others to cells that are on their way to the fully contracted state, and lastly, others that are in the resting condition. With muscle cells in these varying states, the extent of tone will be determined by the ability of the fully contracted cells to move the structure. And feeble and strong tone differ in the number of elements involved, rather than in the extent of activity in the individual elements. (Though this does not imply that some variation in the strength of individual contractions does not exist.)

If this idea of the mechanism of tone is correct it will possibly throw some light on how tone rhythms are produced. For example, if the number of separate elements producing tone is small, or if the transfer of activity from element to element is slow, the overlapping of the maxima of active states may be imperfect, and slight periodic weakenings of tone be produced, giving rise to tone rhythms characteristic of all structures that show this form of activity.[7] In the vessels of the bat's wing and the portal vein we may assume that the rhythmic variations are due to incomplete overlapping of groups of units more or less large. According to the common idea of tone these rhythmic variations are produced by rhythmic alterations in the contractile power of each element of the tissue; a form of activity extremely difficult to explain. My idea of tone implies that the contractile elements of plain muscle producing it act like heart and striped muscle, and probably work on something like the all or none plan.[8] Again, it is possible this idea of tone will throw light on the mechanism producing the form of contraction peculiar to plain muscle when contracting naturally, or as a result of artificial stimulation. In this contraction the muscle shortens slowly and elongates with great slowness. Such tissue, examined histologically at the height of contraction, shows resting and active cells intermingled. So we may be sure not all the elements of the tissue are active at the same time.[9] And it may be possible that in this instance the primary stimulus excites a small number of cells. These become active, and in turn excite another set; the latter work, while the former, now fatigued, rest, but the transmitted stimulus is feebler and the contraction correspondingly weaker. These excite other cells, and so on. The mechanism here is the same as used in producing tone; except that in tone the stimulus does not decline in efficiency. Such a mechanism would give rise to a feeble, long-drawn-out form of activity, like that seen when plain muscle is excited.

[7] Gaskell: Journal of anatomy and physiology, 1817, xi, p. 720; Brunton: *Ibid.*, 1884, v, p. 14; Bottazzi: *Ibid.*, 1897, xxi, p. 1.

[8] Keith Lucas: Journal of physiology, 1909, xxxviii, p. 113.

[9] Keith Lucas: Journal of physiology, 1904, xxx, p. 443, has described the same condition in striped muscle.

METHODS.

Obviously, in order to determine the histological condition of muscle cells producing tone, we must have the power to kill and fix the tissue in any stage of its activity. Devices intended to accomplish this in muscle are few in number, and, in most cases, unsatisfactory. Some investigators have attempted to accomplish this with solutions of hot nitric acid and alcohol. Others have used freezing mixtures, but with indifferent success. This suggested to me that possibly the intense cold of liquid air might be more satisfactory; and its use has demonstrated that for plain muscle it is a remarkably efficient killer. Other killing agents are open to the disadvantage that while killing they act as stimuli. But in liquid air we have an agent capable of stopping the physiological processes in plain muscle practically instantly without stimulation, and possessing the additional advantage of in no wise interfering with the subsequent use of Zenker's or other fixing agent. Tissues killed in liquid air and fixed in Zenker's solution make remarkably good histological preparations. The intestinal and gastric mucosa show cells in an excellent state of preservation. Parts of the pancreas, accidentally included in sections, show zymogen granules in its cells; something that cannot be seen when pancreas is frozen with liquid CO_2. In some respects, then, the freezing with liquid air acts differently from the cold of freezing mixtures and CO_2. It does not seem to disrupt or damage the individual cells. In plain muscle, and some forms of heart muscle, it seems to produce a sort of temporary coagulation of the muscle substance without a contraction. When the ventricle of a turtle is dropped into liquid air it becomes hard and brittle like glass. When it thaws, no contraction is seen, irritability is gone, and the tissue is in a peculiar state. It retains its shape, and is elastic, unlike the normal or dead tissue. Touched, it yields, and when the pressure is removed it springs back to its original shape like a block of stiff gelatin. My method of using the liquid air has been to attach the tissues to delicate levers, and then to freeze the preparations by immersing them in the liquid air, afterward letting them thaw out in cooled Zenker's solution. The method records changes in shape, and fixes the tissue before the effects of liquid air have fully passed off. Such preparations stain beautifully with eosin, haematoxylin, Van Gieson, Mallory's analin blue, etc. When striped muscle, that is, the sartorius of a frog, is immersed in liquid air, it instantly contracts sharply, probably as a result of the stimulation of its nerves, for this contraction is greatly diminished in curarized muscles.[10] It remains in this condition until the muscle is withdrawn from the liquid air and permitted to thaw in the air or in Zenker's. The muscle instantly gives a second remarkably extensive rigor shortening. (This agrees perfectly with L. Herman's description of the action of cold on muscle.[11]) I hope in a future paper to discuss this point more fully. It is only necessary here to call attention to the marked difference between striped and smooth muscles in this respect. The differences are so great that it must seem that these two structures are more fundamentally unlike than has been supposed; because there is no doubt that plain muscle is not so excited when treated with liquid air, and it does not shorten on thawing. As proof of this, we can remove a piece of muscle from the stomach wall of necturus, spread it out flat on a glass plate, pour liquid air over it until it is thoroughly frozen and let it thaw; and there is no curling up of the edges, crinkling, or any change of position as a result of such treatment. Again, if a strip is suspended, attached to a delicately poised lever, the strip can be immersed in liquid air and thawed in air or Zenker without any contraction. It is true a slight motion of the lever can be seen when the suspending thread is wet. This can be increased by deeper submersion in the fluid hence it is undoubtedly due to the shrinking of the wet thread and not to any reaction of the tissue. The action of liquid air is so rapid that active tissue can be caught in any stage. In this way it is possible to get and compare two pieces of tissue in strong and feeble tone, or to stop a piece of suspended plain muscle in any stage of its contraction. In all such preparations a characteristic thing is the presence of active and inactive cells in the tissue, whatever its physiological condition. It is difficult to understand how this could be if the common idea of tone is correct. In feebly contracted muscle, and in structures showing feeble tone, fully contracted cells can be found mingled with cells in intermediate stages. In strongly contracted muscle and sphincters where tone is increased by artificial stimulation, there can always be seen resting cells mingled with ones that are fully active, and others in intermediate stages. The attempts to get preparations of the walls of blood vessels have not been satisfactory. But such as I have, show the same state of the cells, and we conclude the conditions there must be the same, especially since Forster and Henneberg (*loc. cit.*) have both described the presence of active and inactive cells in their preparations of such tissues. It has been suggested that the idea advanced in this paper is based upon an artifact. This is improbable, for the idea is founded upon the existence in active tissues of *inactive* structures, and, as is well known in muscle preparations, the difficulty is to get the inactive forms. Artifacts we would expect to have the form of abnormally active cells. If the idea were based upon active structures it might be open to this objection.

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[10] Folin: This journal, 1903, ix, p. 374, seems to have failed to see this shortening, and he apparently thinks the frozen muscle is in a state of rigor, whereas it is the thawing of muscle that gives its rigor.

[11] Herman, L.: Archiv für die gesammte Physiologie, 1871, iv, p. 188.

CONCLUSION.

The idea that tone is produced by a state of partial continuous activity is unsatisfactory. It is not in harmony with what is known of normal physiological processes in muscle glands and other tissues, and it postulates a peculiar form of activity, differing, in certain respects, from the common modes of acting.

If the facts recorded have been correctly observed, then an interpretation of them would indicate that tone is produced by the same form of activity shown by muscle cells in ordinary forms of activity. It is attended by fatigue in the elements involved, but shows a uniformity of action because only a part of the tissue is involved at one time, and when one element is fatigued, a resting one takes its place. Tone rhythms are produced by imperfect correlation between active parts.

THE INFLUENCE OF THE REMOVAL OF FRAGMENTS OF THE GASTRO-INTESTINAL TRACT ON THE CHARACTER OF NITROGEN METABOLISM. — III. THE EXCISION OF THE STOMACH.

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THE process of nutrition is accomplished in three distinct phases, those of digestion, of absorption, and of assimilation. Each of these contributes an equally important share in maintaining the integrity of the adult or permitting the growth of the young. The part played in this process by the different fragments of the digestive organs has been the subject of numerous investigations at all times. However, most of the efforts thus far were directed to the study of the first two phases. In recent years valuable contributions have been made regarding the laws governing these phases of the process and regarding the mechanism by which they are accomplished. It was found that the stomach played a subordinate rôle, both in the process of digestion and absorption, and on this ground there developed a tendency among physiologists to attribute to this organ a very secondary part also in the process of assimilation.

In a previous communication by Levin, Manson, and Levene,[1] on the nitrogen metabolism after gastroenterostomy, the observation was brought to light, that after this operation the power of the animals to retain and to assimilate the nitrogen of the food was diminished. The animals maintained their nitrogenous equilibrium, but all the nitrogen ingested in excess over the standard diet was removed within the first twenty-four hours following the intake. It should be added here that in the review of the literature on the subject given in that communication, several older publications regarding clinical observations on metabolism after gastroenterostomy in man were omitted.

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[1] LEVIN, MANSON, and LEVENE: This journal, 1909–10, xxv, pp. 231–253.

They are those of Heinsheimer,[2] Joslin,[3] and Deganello.[4] The results obtained by these writers are in harmony with the view expressed in the first article of this series. They all contain records showing that after gastroenterostomy nitrogen retention is either totally absent or is very insignificant. It was realized at the time of that publication that more convincing information regarding the rôle of the stomach in the process of assimilation could be obtained through the study of protein metabolism on animals after complete gastrectomy.

It has been known since the time of Czerny and Kaiser[5] that man and animals may survive this operation and remain in apparently good health. However, there are recorded comparatively few investigations of the nitrogen metabolism after excision of the stomach. The few reported observations[6] were made on several persons operated for carcinoma. According to these reports patients after the extirpation of the stomach not only were capable of maintaining nitrogenous equilibrium but at times retained part of the ingested nitrogen. On the basis of these observations the authors reached the conclusion that normal metabolism is restored in man after gastrectomy. However, the conditions under which the work was done did not always permit of a desired thoroughness, and therefore the conclusions are not very convincing. Besides, it is necessary to bear in mind that the operation of gastrectomy was performed on individuals who suffered from a mechanical difficulty in receiving nourishment, and in whom the stomach was the focus of a diseased process and a source of intoxication. Thus the patients had been, for a long time previous to the operation, in a condition of under-nutrition, or even in a state of partial inanition. After the operation the patients are in a state of reconvalescence when the power of regeneration of the tissues is exaggerated. This consideration may serve to explain the peculiarity of the behavior of the patient under the observation of Deganello. On a diet containing 24.14 gm. of nitrogen, in four days the patient retained 3.03 gm. of nitrogen. This surely indicates a very unusual capacity for nitrogen retention. In considering the records of the patients with gastrectomy, it is also important to bear in mind that there exists no evidence that the removal of the stomach was complete.

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[2] HEINSHEIMER, F.: Mittheilunge aus der Grenzgebieten der Medizin und Chirurgie, 1895, i, p. 348.

[3] JOSLIN: Berliner klinische Wochenschrift, 1897, p. 1047.

[4] DEGANELLO, N.: Archives italiennes de biologie, 1900, xxxiii, p. 132.

[5] CZERNY: Beiträge zur operativen Chirurgie, 1878.

[6] WROBLEWSKI: Centralblatt für Physiologie, 1897, xi, p. 21; HOFFMAN: Münchener medicinische Wochenschrift, 1898, p. 560; DEGANELLO: *Loc. cit.*

The observations on animals as compared with those on men are not more satisfactory either in number or in character. Carvallo and Pachon [7] report a successful operation on a cat. The animal gained in weight immediately after the operation from 1,850 gm. to 2,255 gm. But, after some time, the condition of the animal grew very serious; it refused food and died six months after the operation. The observations of De Fillipi [8] on a dog are also very imperfect.

In the present investigation it was intended to follow the plan described in the two previous communications of this series,[9] but many difficulties presented themselves in placing the animals in nitrogenous equilibrium, in maintaining the uniform nitrogen output through all periods of the day, etc. Finally it was concluded to plan the principal experiments in periods lasting four or five days. Nevertheless, the catheterization of the animals was performed every twenty-four hours and the feeding took place at short intervals (every hour, ten times a day). Autopsies performed on the animals at the close of the experiments have revealed that in one of the animals a very small part of the pyloric end — not more than 15 mm. in length — still remained unexcised; in the second the resection was complete.

In the second animal at no time was there observed any nitrogen retention after the equilibrium was once established; in the first animal, immediately after the operation a marked retention of nitrogen could be observed, and only after some time, especially after the mode of feeding had been changed, the animal removed in twenty-four hours all the nitrogen taken in excess over the quantity required for the maintenances of the established equilibrium. An attempt to interpret the observations in detail will be given below; here it suffices to state that the results of the experiments on dogs with gastrectomy confirm the view previously advanced on the rôle of the stomach in the process of protein assimilation expressed in the first communication of this series.

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[7] Carvallo and Pachon: Archives de physiologie, 1895, vii, pp. 349–355 and pp. 766–770; also Travaux du laboratoire de Ch. Richet, 1895, iii, p. 456.

[8] De Fillipi: Deutsche medicinische Wochenschrift, 1894, p. 780.

[9] Levin, Manson, and Levene: *Loc. cit.* and also Carrel, Meyer, and Levene: This journal, 1909–10, xxv, p. 439.

PLAN OF EXPERIMENTS.

As soon as the animals recovered from the operation they were placed in a condition of nitrogen equilibrium on a diet containing not less than 70 calories per kilo weight. The food consisted of chopped lean beef, prepared according to the recommendation of W. J. Gies [10] and preserved in a refrigerator, together with plasmon, cracker meal, lard and salt. The attempt to maintain animals on the simpler diet of plasmon, cracker meal, and lard was abandoned, since it was noted that only in absolutely normal animals is this diet palatable for an indefinite time. All the animals that had undergone any one of the operations on the digestive organs took the simpler food only for a short time. This may be worth noting, since it contains perhaps an indication that even after an apparently complete recovery the animals in reality never again regained their normal health and the keenness of their normal appetite.

On the days of the experiments the animals received with the first meal, in addition to their standard diet, a quantity of plasmon equivalent to 1 or 2 gm. of nitrogen. The nitrogen elimination during that period was compared with the nitrogen output of a normal period of the same duration.

METHODS OF ANALYSIS.

The analytical methods employed in this investigation were the same as those described in the previous communication of this series.

EXPERIMENTS.

Dog I., weighing 13.5 kg., operated January 28th. Apparently uneventful recovery. On the 14th of February the animal vomited occasionally. This symptom did not recur again.

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[10] GIES, W. J.: This journal, 1901, v, p. 235.

Experiment I. — On the 28th of February the animal received a diet containing 5.89 gm. of nitrogen and 798 calories. The urine was collected in four-hour periods. On the following day the intake of the animal was raised to 7.82 gm. and 871 calories. Weight of dog, 12 kg.

The output on the two respective days was the following:

	Standard diet. Gm. N.	Standard diet + plasmon. Gm. N.
Intake	5.890	7.890
Output: feces	0.567	0.580
Absorbed	5.323	7.310
Output: urine	3.385	4.490
Balance	+1.938	+2.810

Absorbed in excess over first day	1.987
Eliminated in urine over first day	1.105
Balance between first and second day =	+0.882

Thus there was noted a retention of 44.5 per cent of the excessive nitrogen intake. A normal dog under similar conditions[11] retained 43 per cent of the excessive intake.

Experiment II. — It is seen, however, that at the time of the experiment the animal was not yet in a condition of equilibrium. The diet was modified and on the 13th day of March the animal was in a condition of equilibrium at an intake of 3 gm. of nitrogen and 814 calories. Weight of dog, 12.06 kg.

On March 16 and 22 experiments were performed with a diet containing a quantity of plasmon equivalent to 1.0 gm. of nitrogen in excess over the standard diet. Weight of dog, 11.9 kg.

	Standard diet. Gm. N.	Standard diet + plasmon. Gm. N.
Intake	3.000	4.000
Output: feces	0.480	0.552
Absorbed	2.520	3.448
Output: urine	2.600	2.730
Balance	−0.080	+0.718

Absorbed in excess over first day	0.928
Eliminated in urine over first day	0.130
Balance between first and second day	+0.798

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[11] Levene and Meyer: This journal, 1910, xxv, p. 217.

In these experiments there was noted a higher nitrogen retention than in the normal animal. This result seemed rather paradoxical at the first glance. A possible interpretation of it could be found in the following considerations. Under normal conditions the protein of food enters the duodenum only in form of primary digestion products, in small portions, thus bringing about a perfect digestion of the ingested protein material. After gastrectomy a large quantity of wholly undigested protein reaches the duodenum. The absence of gastric secretion may lead to a very imperfect secretion of the pancreatic juice and perhaps also of erepsin. Thus the protein material under these conditions remains in the intestinal tract for a considerable time undigested, and is absorbed in that form. The fate of such protein in the organism one would expect to be the same as that of parenterally introduced protein. It has been established by recent investigators that parenterally introduced protein is retained in the organism completely, if the animal is maintained in a condition of normal nutrition. The greater retention of nitrogen after gastrectomy, as compared with the retention after gastroenterostomy, observed on man, and in our experiments, may be interpreted in the light of these considerations. It was planned to subject this possible interpretation to further analysis. However, before sacrificing the animal, it was concluded to extend the observations, of the same character as described, to a longer period.

Experiment III. — On April 9 an experiment was begun lasting fifteen days. It was divided into three periods of five days each. During the first five days the intake of the animal contained 3 gm. of nitrogen and 814 calories. The following five days the intake contained 4.0 gm. of nitrogen and 846 calories, and the intake of the last five days was the same as the first period.

The results of this experiment were the following:

	First period, st'd diet.		Second period, st'd diet + plasmon		Third period, st'd diet.	
	Urine N.	Feces N.	Urine N.	Feces N.	Urine N.	Feces N.
	2.40	.478	3.06	.540	2.90	.110
	2.16	...	3.41	.596	3.28	.354
	2.31	.901	3.75	.619	2.55	.717
	2.73	.730	3.62	.583	2.76	.518
	2.54	.540	3.40	.600	2.60	.463
Totals	12.14	2.649	17.24	2.938	14.09	2.162

	First period.	Second period.	Third period.
Intake	15.000	20.000	15.000
Output: feces	2.650	2.938	2.162
Absorbed	12.350	17.062	12.838
Output: urine	12.140	17.240	14.090
Balance	+.210	−.178	−1.252

Absorbed in excess over first period . .	4.712
Output in urine over first period . . .	5.100
Balance over first period	−.388

Thus at this time no retention of the additional nitrogen took place.

Experiment IV. — Another experiment of the same nature was performed beginning April 30. The observations were made during eight days. They consisted of two periods. During the first, the intake of the animal contained 3.0 gm. of nitrogen and 814 calories. During the second, 4.0 gm. of nitrogen and 846 calories.

The result of this experiment was as follows:

	Standard diet.		St'd diet + plasmon.	
	Urine N.	Feces N.	Urine N.	Feces N.
	2.52	.418	2.94	.458
	2.40	.410	3.26	.436
	2.56	.561	3.16	.525
	2.49	.553	3.77	.757
Totals	9.97	1.942	13.13	2.176

	First period.	Second period.
Intake	12.000	16.000
Output: feces	1.942	2.176
Absorbed	10.068	13.824
Output: urine	9.970	13.130
Balance	+.098	+.694

Again, in this experiment no nitrogen retention was observed during the period of additional nitrogen. It is also noteworthy that during the first two weeks following the operation the weight of the animal was continually increasing, while it remained constant after that period.

Thus it is possible that after a time the secretion of digestive glands is adapted to the new condition and the digestion of the ingested protein is completed in the intestines without the aid of gastric secretion. In this phase the animals find themselves in a condition similar to gastroenterostomy and the nitrogen retention becomes minimal. This interpretation seems very plausible. However, the autopsy of the animals revealed another condition which offers a basis for another interpretation for the difference in the fate of the additional nitrogen of the diet at the period immediately following the operation and at a considerably later period. Namely, it was noted on the autopsy that the upper part of the duodenum had undergone a considerable dilatation and its walls a marked hypertrophy. Thus it is possible that this artificial sack served as a mechanism regulating the transfer of the food to the intestines and in this manner rendering intestinal digestion more complete.

Dog II., weighing 14.36 kg., was operated on the 7th of January. Soon after the operation, the animal developed distemper. It slowly recovered health and on the 4th of April it was possible to begin the observations on the nitrogen metabolism of this animal. At that time the animal was in nitrogenous equilibrium.

Experiment I (April 4). — Food contained 5.29 gm. nitrogen and 756 calories; the weight of the animal was 11.1 gm. The following day the animal was given, in addition to the standard diet, a quantity of plasmon equivalent to 1.0 gm. of nitrogen, and on the day following this, again, the standard diet. The output in the three respective days was the following:

	First day. Gm. N.	Second day. Gm. N.	Third day. Gm. N.
Intake	5.29	6.29	5.29
Output: feces	0.66	0.69	1.33
Absorbed	4.63	5.60	3.96
Output: urine	4.72	5.68	4.49
Balance	−.09	−.08	−.53

Absorbed in excess over first day	0.97
Eliminated in urine over first day	.96
Balance between first and second day	+.01

Thus, in this experiment, all the additional nitrogen contained in the food of the second day was removed within twenty-four hours.

Experiment II. — This experiment lasted fifteen days. In course of the first five days, the animal received the standard diet of the first experiment; in the second period, also of five days, the animal received daily, in addition to the standard diet, a quantity of plasmon equivalent to 1 gm. of nitrogen, and during the third period the intake again consisted of the standard diet. The nitrogen balance during each of the three periods was as follows:

	First period, st'd diet.		Second period, st'd diet + plasmon.		Third period, st'd diet.	
	Urine N.	Feces N.	Urine N.	Feces N.	Urine N.	Feces N.
	5.46	.469	6.52	1.010	5.55	.580
	5.90	.990	6.32	1.165	6.08	.790
	5.60	.546	6.13	1.085	6.10	.661
	5.80	.840	6.34	.745	6.00	.432
	5.50	.839	6.49	1.176	6.00	.728
Totals	28.26	3.684	31.80	5.181	29.73	3.191

	First period. Gm. N.	Second period. Gm. N.	Third period. Gm. N.
Intake	26.450	31.45	26.45
Output: feces	3.684	5.181	3.19
Absorbed	22.766	26.319	23.26
Output: urine	28.260	31.800	29.73
Balance	−5.494	−5.481	−6.47

Absorbed in excess over first period . .	3.553
Eliminated in urine in excess over first period	3.540
Balance	+0.013

Thus also in this experiment no retention of nitrogen was observed. It was also noted that notwithstanding the intake to maintain the equilibrium of a normal animal of the same weight, this animal was losing both nitrogen and weight.

Experiment III. — This experiment was performed in order to test the correctness of the assumption that the very high rate of nitrogen retention observed in the first dog in the experiments performed soon after the operation was occasioned by the absorption into the circulation of the unchanged protein.

The animal at the time was receiving a diet containing 5.29 gm. of nitrogen and 900 calories. On the 29th and 30th of April the animal received a subcutaneous injection of 191 c.c. horse serum containing 3.09 gm. of nitrogen. The serum had been previously heated to 60° C. for thirty minutes. The nitrogen balance two days previous to the injection and on the day of the injection was the following:

	St'd diet.	St'd diet + horse serum 111 c.c. = 1.21 gm. N. subcutaneously.
Intake	5.29	6.50
Output: feces	.607	.624
Absorbed	4.683	5.876
Output: urine	6.050	6.25
Balance	−1.367	−.374

Thus all the nitrogen received by the animal parenterally was retained in the organism in the same manner as the nitrogen of the additional diet in the early experiments on the first dog.

Experiment IV. — This experiment lasted six days and consisted of two three-day periods. During the first period the diet contained 5.29 gm. of nitrogen and 900 calories; during the second period it contained additional plasmon equivalent to 1.0 gm. of nitrogen. The nitrogen balance of the two periods was the following:

	First period, standard diet.		Second period, st'd diet + plasmon.	
	Urine N.	Feces N.	Urine N.	Feces N.
	4.80	.410	5.87	.539
	4.80	...	5.64	.369
	4.90	.815	5.85	.486
Totals . . .	14.50	1.225	17.36	1.494

	First period.	Second period.
Intake	15.870	18.870
Output: feces	1.225	1.494
Absorbed	14.645	17.376
Output: urine	14.500	17.360
Balance	+0.145	+.016

Thus in the three experiments on the second dog no nitrogen retention was observed. The fate of the additional nitrogen intake in this animal was the same as in the dogs with gastroenterostomy, and the same as in the later experiments on the first animal with gastrectomy. In order to interpret the divergence in the early experiments on the first and the second animals it must be borne in mind that the observations on the second animal were begun on the twelfth week after operation; that in the first animal, in the experiments performed at the lapse of only ten weeks after the operation, already no nitrogen retention was observed after the additional intake. On the other hand, the autopsy of this animal revealed that the extirpation of the stomach on this animal was complete.

We are indebted to the kindness of Dr. E. L. Opie for the following report on the autopsy: — The mucous membrane is 2 mm. in thickness, the villi appear to be considerably larger than normal and the glands longer; otherwise, the tissue appears to be normal.

SUMMARY.

1. Observation on the fate of the nitrogen intake received in addition to that of the standard diet was studied on two animals with gastrectomy.

2. In the first animal there was noted a high nitrogen retention in the experiments performed early after the operation, and no retention in the experiments performed after the tenth week following the operation.

3. The observations on the second animal were begun on the twelfth week after the operation. Three experiments were performed on the animal and no nitrogen retention noted in any one of them.

4. The autopsy revealed that in the first animal a small part of the pyloric end, not more than 15 mm. in length, remained unexcised. The extirpation of the stomach in the second animal was complete.

5. Parenterally introduced protein (in addition to the standard diet) was completely retained in the organism.

6. An analysis was made of the possible conditions which might have occasioned the divergence in the behavior of the additional nitrogen in the experiment following early after the operation and those after a lapse of about ten weeks.

7. The view was analyzed that immediately after the operation not only the gastric secretion was missing, but also the pancreatic and in-

testinal secretions were minimal, and that at that period the absorbed protein had the character of parenterally introduced protein; and that at a later period the pancreatic and intestinal digestion were restored to their normal power.

8. The autopsy revealed a hypertrophy of the upper end of the duodenum developing after the operation.

ON THE PHARMACOLOGICAL ACTION OF URANIUM.

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THE various compounds of uranium have never occupied a prominent place in the field of practical therapeutics. The element possesses marked toxic properties, and but few scientific suggestions have ever been made concerning the utilization of any of its pharmacological actions in the treatment of disease.

A brief survey of the literature is sufficient to convince one that the physiological actions of this substance have by no means been completely determined. Perhaps the most complete and satisfactory study of the physiological action of uranium which has yet appeared was published in 1890 by Woroschilsky.[1] Valuable work was also done by Chittenden[2] and his colaborers in 1885–86. Recently some attention has been given to uranium as a convenient agent for the production of certain forms of experimental edema.[3]

This article will deal with only three general phases of the pharmacological action of this metal, *viz.*, first, the action of the substance on the secretion of lymph; second, its action on the circulation and respiration, and third, its action on the blood.

If sodium uranate be added to a strong aqueous solution of tartaric acid, a soluble compound of uranium will be formed which may be used for intravenous injections without precipitation of the blood. It is usually necessary to add a small amount of sodium hydrate in order to completely neutralize the tartaric acid after the desired amount of sodium uranate has been added. It is, however, probably slightly more convenient to proceed as did Woroschilsky in making the double salt. Nearly all of the following experiments have been performed with solutions made up after this method. It consists in heating uranium nitrate in a crucible until all the acid is driven off and the reddish-brown oxide (UO_3) has been formed. The oxide is then carefully weighed and from this weight the percentage strength of the solution is calculated. The oxide is placed in a small amount of distilled water, heated, and small quantities of tartaric acid are added from time to time until the oxide is dissolved. The slight excess of tartaric acid which is usually found to be present is neutralized with sodium hydrate, using phenolphthalein as an indicator. The solution is then diluted with distilled water until the desired percentage is obtained. The neutralized solution is of a bright golden-yellow color. I have used in nearly all cases a 2 per cent solution of the red oxide (UO_3), the percentage being based on the relation of the weight of the oxide used to the amount of the solution when changed into the double salt (sodium uranium tartrate) and completely diluted. A solution made up in this manner gives no visible precipitate of egg albumen, blood nor serum when added to either of these proteid solutions in a test tube. It possesses moderate antiseptic power, but some fungi grow readily in 2 per cent solutions of it. If the solution be made slightly acid, it gains greatly in antiseptic power and will also precipitate proteids. Addition of a little sodium hydrate will again dissolve the proteid precipitate which is also soluble in an excess of the albumen.

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[1] Woroschilsky: Arbeiten des pharmakologischen Instituts zu Dorpat, 1890, v, p. 1.

[2] Chittenden: Studies from the Sheffield Scientific School, 1885–86, i, ii, iii.

[3] Pearce: The archives of internal medicine, June, 1909.

The administration of sufficient quantities of uranium to an animal is usually followed within a few days by albuminuria, glycosuria, parenchymatous degenerations, gastro-intestinal disturbances, paralyses, and, according to most observers, certain marked pathological changes are found in the blood vessels.

Action on lymph flow: — The effect of uranium on lymph flow may best be described by the following protocol of one of my experiments.

Feb. 18, 1910. — Dog, male, yellow, weight 18 kilos, in good condition. Twelve hours previously the animal had eaten some meat, no other solid food taken before the experiment. Etherized and placed upon the operating table. Arrangements made for artificial respiration when needed. Blood pressure taken from right carotid artery, injections made into the left femoral vein. Chest opened a little at the apex and a cannula placed in the thoracic duct. Normal lymph flow was 37 drops in fifteen minutes, *i. e.* 12⅓ drops in each five minutes. Normal time required for clotting of the lymph was four and one-half minutes. Lymph was thin and somewhat opalescent, *i. e.* fat was present but not abundant.

11.22. Injected 10 c.c. of a 2 per cent (UO_3) solution of sodium uranium tartrate. In the next five minutes 12 drops of lymph were secreted.

11.28. Injected 10 c.c. of drug. In the next five minutes 16 drops of lymph were secreted.

11.34. Injected 10 c.c. of drug. Lymph flow 13 drops in five minutes.

Lymph clots readily after 30 c.c. of drug had been injected. Hence not more than traces of the uranium could be present in an active form in the secreted lymph, for the addition of a very small amount of the drug to the lymph outside the body prevents clotting.

11.42. Blood pressure fallen about one half. Muscular tremors well marked but weaker than with eserine. Lymph remains clear and opalescent. No noticeable increase in the rate of flow.

11.56. Lymph still clear, no traces whatever of any blood streaks.

11.57. Injected 30 c.c. of drug to kill the animal. Urinated, convulsions, tremors. Slight increase and then a decrease in rate of lymph flow.

12.00. Artificial respiration stopped and animal died of asphyxia. Was still in fair condition just before death.

In this case observations upon the lymph flow were continued for only about three quarters of an hour, but the results are perfectly typical of those obtained in other cases in which observations were carried on for a much longer time. In some instances a very slight increase over the normal is obtained. I believe this to be due not to any specific action of the drug on the formation of lymph but rather to the muscular movements of the visceral organs, and in case of convulsive contractions of the skeletal muscles in general these probably also help to force a few extra drops of lymph out of the thoracic duct or its immediate branches. The increased muscular movements are probably partly due to a slight stimulation of the motor endings in striated muscle. There seems also to be a clearly defined but [illegible] certain parts of the central nervous system [illegible] marked depression and paralysis [illegible] were seen in the [illegible] In [illegible] the quantity of blood [illegible] increased progressively in amount as the intoxication [illegible] however [illegible] accompanied [illegible] the rate of [illegible] flow was [illegible] extent [illegible] sufficient [illegible] when he gave them [illegible] water [illegible] He assumed that the [illegible] and concluded that the [illegible] probably injury to the capillary [illegible] of water was sufficient to produce [illegible] rabbits. His experiments extended over a [illegible] probable that in that interval pathological [illegible] which would be entirely absent [illegible] producing in a relatively short period [illegible] present instance I have not attempted [illegible] that [illegible] immediate action of the drug. [illegible] however [illegible] animals [illegible] within an hour or so [illegible] hyperemia [illegible] probable that [illegible] at least partly due to a slight, continuous, but gradually increasing stimulation of the vaso-constrictor centre in the medulla. This stimulation is probably due in part to a sort of compensatory (asphyxial) action by which the circulation tries to counterbalance the gradually progressing depression of the respiratory centre. The increased volume of blood caused by the rapid introduction of 8 or 10 c.c. of fluid into the circulation usually produces a slight rise of blood pressure lasting about as long as the injection continues. I have observed that this elevation is often followed by a slight fall which is also transient and may amount to 20 or 25 mm. (mercury manometer) (Fig. 1). I believe this fall is mainly due to a direct depressant action on the heart, for it also occurs after section of the vagi, and sometimes I have noted after large injections that the heart may weaken and miss one or two beats and then gradually regain its normal strength. This may also occur after section of the vagi. Seven or 8 c.c. of a 2 per cent solution (UO_3 changed to the double salt)

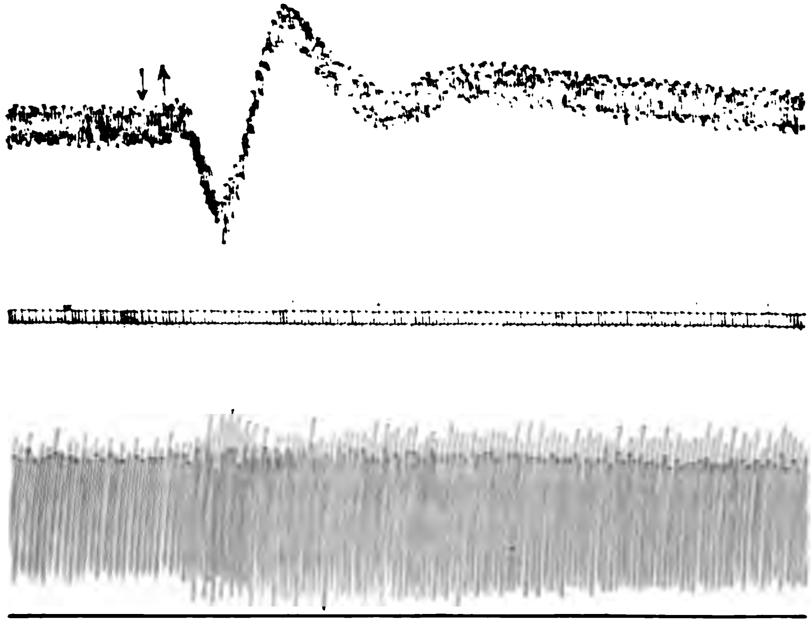

FIGURE 1.—Carotid blood pressure (with mercury manometer) and respiratory tracings from a dog. The middle curve is the time in seconds. The straight line marks atmospheric pressure. At the arrows 8 c.c. of sodium uranium tartrate (2 per cent) were injected.

generally produces a rise of about 50 mm. of mercury in a medium-sized dog. Electrical stimulation of a vagus nerve stops the heart after repeated large injections of the substance. Incidentally it may be mentioned that the secretory endings of the chorda tympani in the submaxillary gland, the endings of the phrenics and the motor endings of the sciatic remain active throughout the intoxication. Injection of 2 per cent solution of uranium nitrate causes a marked transient fall in blood pressure which could scarcely be distinguished from the action of amyl nitrite. Uranium acetate also causes a lowering of pressure. Disodium tartrate caused no effect upon the circulation aside from the increased volume of fluid in the vessels.

Action on the respiration. — Injection of moderate amounts (7 to 10 c.c.) of 2 per cent solution of the double salt caused a very slight increase in both rate and depth of respiration (Fig. 1). The immediate cause of death is respiratory paralysis. Uranium has long been supposed to possess an action somewhat resembling that of hydrocyanic acid.[6] I find its stimulating effect upon the respiration to be much smaller than that of the cyanides. Early in its action the depression of the respiratory centre becomes evident. After a time the respiration ceases but the circulation remains in good condition and if artificial respiration be given the animal may be kept alive for a long time. Normal respiration does not return until after ten to thirty minutes if it is reinstated at all. The cessation of the breathing is usually rather rapid and there is but little tendency toward Cheyne-Stokes respiration as is often seen with the cyanides. I have repeatedly noticed when the respiration had become greatly depressed that the injection of another dose of the substance would tend to revive the animal, apparently very much in the same way as the cyanides. This is probably due mainly to a slight direct stimulation of the central nervous system, for the effect comes on immediately after injection of the drug. The remarkable specific depression which this substance exercises on the respiratory centre reminds one of morphine. I have regularly observed that after a certain amount of the drug had been administered the animal would pass into a sort of comatose condition in which but little ether would be required to maintain the anæsthesia.[7]

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[6] Kobert: Ueber Cyanmethaemoglobin und den Nachweis der Blausäure, Stuttgart, 1891, Lehrbuch der Intoxicationen, 2d ed., pp. 94–99; Geppert: Zeitschrift für klinische Medicin, 1895, xv, pp. 208 and 307.

[7] Meltzer: This journal, 1908, xxviii, p. 141.

This may be due to the gradually progressing central paralysis, but it may also be contributed to, in part at least, by some specific action on the blood. The view has been generally held of late years that uranium retards the reduction of the oxyhæmoglobin in the tissues. I shall refer to this point again.

Action on the blood. — Uranium and the cyanides both prevent coagulation when added in sufficient quantities to drawn blood. Is this action the same in each case? I have attempted to throw some light on this question by trying the action of the drugs on certain common ferments.

If an aqueous extract of potato peelings be treated with a small amount of tincture of guaiac, an oxidizing ferment from the potato will at once oxidize the guaiac over to a blue compound. If a uranium solution (either the acetate, nitrate or double salt) be added to the aqueous extract before the tincture of guaiac is poured in, there will be no hindrance whatever to the normal change over to the blue compound. Apparently uranium exercises no inhibitory power at all upon this ferment. If a solution of potassium cyanide be added to the aqueous extract before the tincture of guaiac is added, then no blue color will appear at all. Evidently hydrocyanic acid acts differently from uranium upon this ferment.

When normal blood is treated with hydrogen peroxide, a marked evolution of gas is at once produced. If a solution of uranium (either the acetate, nitrate or double salt) be added to the blood before the hydrogen peroxide is added, the formation of gas is in no wise hindered. Apparently the reaction takes place exactly the same as in normal blood. When blood is treated with a cyanide and then hydrogen peroxide is added, it is generally stated that no gas at all is produced. As a matter of fact a very little gas is usually formed, but the reaction is entirely different from that produced by the hydrogen peroxide on normal blood or in the presence of uranium. Evidently uranium and hydrocyanic acid manifest different properties so far as this reaction is concerned.

If normal blood be treated with a little tincture of guaiac, a yellowish mixture is obtained but no blue compound is produced. Addition of a little hydrogen peroxide to the mixture at once causes a great evolution of gas and the development of a deep blue color. The presence of some of the earlier formed yellowish substance gives the whole mixture a deep greenish-blue appearance. If the experiment be repeated in the same manner but with the addition of a little uranium solution to the blood before the hydrogen peroxide is added, a profuse evolution of gas and the formation of a deep greenish-blue color is at once observed. The reaction appears to take place exactly as it would if no uranium had been added. When a cyanide is substituted in place of the uranium in the above experiment, no gas is formed but the deep greenish-blue color at once makes its appearance. Evidently in this case, so far as the development of the blue compound is concerned, neither uranium nor the cyanides exercise any noticeable inhibitory activity. The formation of the blue compound in this case seems to be separate and independent from the formation of the methæmoglobin. Incidently these experiments show that the ferment in potato peelings which causes the guaiac to be oxidized to the blue compound is by no means so resistant as the corresponding ferment in blood, for cyanides stop the action of the former and not that of the latter.

The general results of these experiments may be summed up in a few words. Cyanides and uranium act differently on some ferments but apparently have the same or possibly exert almost no action on others. Unfortunately these conclusions cannot throw any light upon the question of the similarity or the difference in the methods by which cyanides and uranium prevent the coagulation of blood. It is to be noted, however, that uranium does not inhibit the actions of these ferments.[8]

It was shown by Collingwood[9] that if a 2 per cent solution of disodium hydrogen phosphate (Na_2HPO_4) be added to blood (in any amount up to an equal volume) and the free calcium ions be thus precipitated out, the blood would still clot, but a somewhat longer time (one half hour) was required for the completion of the process than was necessary in the case of normal blood. The usual test for uranium is the formation of a precipitate when a phosphate solution is added to a solution containing uranium. It seemed that this coincidence might furnish an opportunity to determine something concerning the action of uranium. Since sodium uranium tartrate does not precipitate dilute calcium chloride solutions, we would expect some sort of proteid combination to be formed by the uranium, or the presence of the metal may in some way check the action of the ferments. At any rate, if it should be possible to add sufficient phosphate to precipitate all the free calcium in the blood (and still leave an excess of phosphate ions free (?) in the plasma), then the addition of an equal volume of a 2 per cent solution of disodium hydrogen phosphate to blood which has been prevented from clotting by the previous addition of the smallest effective quantity of uranium might cause clotting to take place, this, however, being dependent upon the nature of the uranium action. If it were merely inhibiting the ferments by its presence in solution, clotting ought to occur. If it were loosely bound to some proteid element, clotting would probably occur. If it should be firmly combined with some proteid absolutely essential to clot formation, clotting would probably not occur.

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[8] CHITTENDEN: *Loc. cit.*, found in general that an extremely small amount of uranium increased but more decreased the activity of the digestive ferments, the reactions varying somewhat with the different uranium salts used.

[9] COLLINGWOOD: Journal of physiology, 1909, xxxviii, Proceedings of the Physiological Society, p. lxxix.

A series of experiments were carried out to test this point. Perfectly fresh blood was obtained from etherized dogs and was used immediately after withdrawal from the right femoral artery. It was found that if a small amount (one tenth volume of 2 per cent solution) of sodium uranium tartrate be added to fresh blood and clot formation be thereby prevented, then the addition of a 2 per cent solution of disodium phosphate (Na_2HPO_4) in any amount up to one half of the total volume would not cause clotting to occur. Nor would the addition of calcium chloride solution cause the blood to clot, either before or after the addition of disodium phosphate. This would indicate that either the ferments or some of the proteid elements of the blood had been affected by the uranium and that its action is different from that of the oxalates, for the careful addition of a slight excess of calcium chloride causes oxalated blood to clot. Again, the action of uranium in preventing clot formation differs from that of the fluorides in at least two particulars. The addition of a small amount of thrombokinase derived by aqueous extraction from minced spleen and lymphatic glands causes blood treated with potassium fluoride to clot in almost the normal time. The addition of thrombokinase is entirely without effect upon blood treated with uranium. Also, if a small excess of calcium chloride be added cautiously to blood treated with a fluoride solution, clotting will occur. As stated above, addition of calcium does not cause clotting in blood containing uranium. It was supposed by Rettger[10] that the fluorides act by combining with the calcium to form calcium fluoride, which in turn is bound in some way to a portion of the protein. It may be that uranium acts in some such way, but it is to be noted that blood treated with uranium and blood treated with fluorides react in an entirely different manner toward calcium and thrombokinase. It may be further mentioned that dialysis causes fluoride blood to clot. I was unable to detect the formation of any true clot in uranium blood after twenty-four hours of dialyzing in running (tap) water. Uranium acts differently from magnesium sulphate inasmuch as the addition of thrombokinase causes clotting in blood treated with that salt. And further, magnesium sulphate blood may be made to clot by sufficient dilution with salt solution. No amount of dilution will cause clotting in blood treated with uranium.

It might be questioned as to whether or not disodium phosphate would precipitate out all the uranium when added to blood which had been treated with that metal. If to a Ringer's or similar salt solution to which a small amount (one tenth volume of 2 per cent solution) of sodium uranium tartrate has been added there be further added a slightly larger quantity (one fourth volume of 2 per cent solution) of disodium hydrogen phosphate, a marked precipitate will soon be produced. Mainly calcium phosphate comes down at first, but after a little time it appears that the uranium also is precipitated, for if blood be added to the clear filtrate clotting will occur in approximately the normal time. And if the precipitated calcium and uranium phosphate be washed with a little distilled water, dried and then after comminution with a little normal salt solution be added to blood, clotting will occur in about the normal time. It would appear, then, that pure uranium phosphate is sufficiently insoluble in blood to be unable to prevent clot formation. (This also holds good for sodium uranate which is insoluble.) It should be stated that when a solution of disodium hydrogen phosphate is added to a solution of sodium uranium tartrate the uranium phosphate is not precipitated immediately but some ten to twenty minutes are required for complete precipitation. Consequently when disodium hydrogen phosphate is added to blood previously treated with uranium it is probable that complete precipitation would not occur for some twenty minutes or longer. But if after a period of twenty or thirty minutes either calcium chloride, thrombokinase, fibrin ferment (serum from blood clot) or any two (or all three of these) be added, no clot will be formed. The addition of fibrin ferment would seem to indicate that the fibrinogen had been affected, for if all the free soluble uranium has been precipitated then the addition of fibrin ferment should produce clotting unless the fibrinogen has been affected. It seems probable, however, that the uranium when first added to the blood rapidly forms combinations with practically all of the proteid elements at hand and that even if the excess of free uranium (provided any such quantity should be left over) is later precipitated out by disodium hydrogen phosphate, then the uranium proteid compounds are not broken up but still maintain practically their original condition. These compounds are apparently also soluble in excess of proteid, for if a small amount (2 c.c.) of the blood treated with phosphate after uranium be added to a larger quantity (6 c.c.) of fresh blood and the mixture well shaken clotting will be prevented.

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[10] Rettger: This journal, 1909, xxiv, p. 406.

It may be objected that in the presence of soluble proteids and dilute salt solutions, such as constitute blood, the disodium hydrogen phosphate would not precipitate out the uranium in the usual manner. But if uranium be added to dilute salt solutions approximating as nearly as possible the quantity and quality of those found in the blood, then the disodium hydrogen phosphate will precipitate the uranium so completely that the clear filtrate exercises no noticeable inhibitory action upon clot formation in fresh blood. If such precipitate is not formed when uranium, and then phosphate, are added to blood, it would appear to be because the uranium has already entered into some sort of combination with the proteids themselves. This is what I believe actually occurs. In this connection may be recalled the action of oxalates which precipitate the free calcium of the blood but apparently do not form proteid combinations, for addition of a slight excess of calcium chloride again establishes the conditions requisite for coagulation of the blood. It must, of course, not be forgotten that the addition of a 2 per cent solution of the disodium hydrogen phosphate (in any amount up to an equal volume) to normal blood does not prevent coagulation.

It is generally assumed that the cyanides prevent coagulation by inhibiting the action of the ferments concerned in clot formation. If this be the whole truth (which I am inclined to doubt), then the above results would seem to indicate that the action of uranium is much more extensive than that of the cyanides so far as the inhibition of clot formation is concerned. And uranium certainly acts differently from many of the other inorganic compounds which are ordinarily used to prevent clot formation.

When a cyanide is added to drawn blood outside the body, a bright red color is at once produced. This is usually described as being the same color as that possessed by pure arterial blood. In a similar manner if a small quantity (one-twentieth volume of 2 per cent) of sodium uranium tartrate solution be added in the presence of oxygen or air to freshly drawn blood, this blood will also rapidly take on a bright red color resembling that of arterial blood. To the naked eye cyanide blood and uranium blood look very much alike. It is to be remembered, however, that retinal fatigue, and the constant color variations which are generally to be readily observed in freshly drawn blood when exposed to the air (and particularly if shaken or stirred) render close color distinctions in such cases as this very difficult to make. It would appear that the presence of a certain amount of available oxygen is absolutely necessary for this color reaction to take place when the blood has been treated with uranium. In order to test this point I tried the following experiment. In an etherized dog the external jugular vein was dissected out and clamped with a serresfin. The venous blood (dark) soon accumulated in the vein above the serresfin. A small glass-barrelled hypodermic syringe was then partly filled with uranium solution, care being taken to exclude all air bubbles. The point of the syringe was then passed into the swollen vein and blood was drawn directly into the syringe until the barrel was three fourths full. The point was then withdrawn and a little of the uranium solution was again drawn into the syringe. In this manner but very little opportunity was afforded for air to enter the syringe, which was filled with dark venous blood diluted with uranium solution. It was found that so long as no air entered the syringe the blood remained dark and very venous in appearance. But so soon as the contents of the syringe were emptied out into an open test tube and well aerated the bright red arterial color at once appeared.

This experiment is significant inasmuch as it would appear to indicate that oxygen can readily pass through the walls of the red corpuscles and form a combination with the hæmoglobin within. Since it is not possible to detect any difference spectroscopically between normal blood and blood to which uranium has been added, it therefore becomes difficult to see how uranium could retard the reduction of oxyhæmoglobin so far as the blood itself is concerned. For if oxygen can readily pass into the corpuscles, and no special combination between the uranium and the hæmoglobin is formed, then it would seem that the oxygen might also readily pass out of the corpuscles again.

The bright red color which blood treated with uranium assumes in the air is not appreciably affected by the addition of disodium hydrogen phosphate. It seems probable that the retention of this color is partly, at least, due to changes in the proteids of the red cells. For the color of laked blood is not materially influenced by the addition of uranium.

If a sample of fresh blood be treated with uranium and diluted to a 1 or 2 per cent solution and left standing in a stoppered bottle, it will become dark and show decomposition within one or two days. If a similar sample of blood be treated with a solution of potassium cyanide of corresponding strength and then be similarly diluted and left standing, it will retain its bright red color for several weeks.

It seems that no chemical combination whatever is formed between uranium and the hæmoglobin of the blood. In order to test this I made a long series of observations both with the spectroscope and with the diffraction grating. In the latter case photographic records were made.[11] I was unable to detect by either method any difference between normal blood diluted to 1 per cent or ½ per cent and blood which had been first treated with uranium and then diluted to 1 or ½ per cent. A comparison was also made between blood treated with uranium and blood to which potassium cyanide had been added.

The spectrum of cyanhæmoglobin differs but little from that of oxyhæmoglobin, but a longer time is required for its reduction by ammonium sulphide. Perhaps the reduction of blood treated with uranium may be slightly retarded, but considering the difficulty attendant upon the determination of the completion of the reduction process one would scarcely be justified in saying that the time was prolonged.

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[11] I am greatly indebted to Prof. R. R. Ramsey of the Department of Physics of Indiana University for much valuable assistance in making the photographic observations.

If a sample of methæmoglobin be made by the addition of either iodine or potassium ferricyanide to fresh blood and then the mixture be diluted to a 1 per cent solution, the addition of potassium cyanide will at once cause the formation of cyanmethæmoglobin,[12] the spectrum of which very closely resembles that of reduced hæmoglobin. But if to a dilute solution of methæmoglobin, which has been made by the addition of either iodine or potassium ferricyanide to fresh blood, there be added a solution of sodium uranium tartrate, no change whatever can be made out in the spectrum of the methæmoglobin.

This seems to show that uranium does not form any combination with methæmoglobin, and it certainly indicates that the action of the metal is different from that of the cyanides so far as the hæmoglobin of the blood is concerned. The addition of disodium tartrate does not produce any change in the spectrum of normal blood.

CONCLUSIONS.

1. The intravenous injection of a solution of sodium uranium tartrate in any quantity up to the lethal dose does not produce a noticeable increase in the rate of lymph flow from the thoracic duct in the dog.

2. The rise in blood pressure produced by the intravenous injection of a solution of sodium uranium tartrate into a dog is of a much more pronounced and prolonged character than the rise produced by injection of a corresponding quantity of a cyanide.

3. The stimulating action which uranium exercises upon the respiration is vastly less vigorous than that manifested by the cyanides.

4. The method by which uranium prevents the coagulation of blood appears to be different from that exercised by most other substances, and probably consists in the formation of a close direct combination between the metal and some one or more of the proteid elements of the blood which are essential to the process of coagulation. Neither the addition of thrombokinase, fibrin ferment, nor calcium chloride

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[12] KOBERT: MALY's Jahresbericht, 1891, p. 443; HALDANE: Journal of physiology, 1900, xxv, p. 230.

to blood previously treated with sodium uranium tartrate will cause clotting to occur. Nor is it possible to bring about clot formation in such blood by precipitating the uranium with disodium hydrogen phosphate.

5. Neither the spectroscope nor photographic records made by means of the diffraction grating reveal the formation of any chemical combination between uranium and the hæmoglobin of the blood. This is a variation from the action of the cyanides which form cyanhæmoglobin when added to solutions of hæmoglobin.

6. If a solution of sodium uranium tartrate be added to a solution of methæmoglobin, no change whatever occurs in the spectrum of the solution. If, however, a cyanide be added to the methæmoglobin, cyanmethæmoglobin is at once formed.

7. The cyanides prevent the formation of a blue oxidation product from tincture of guaiac by the oxidizing ferments present in an aqueous extract of potato peelings. No such inhibitory action is exercised by uranium upon this reaction. The cyanides also prevent the evolution of gas when hydrogen peroxide is added to blood to which a small amount of cyanide has previously been added. But no inhibition is exercised upon this ferment action by uranium.

8. It seems extremely probable that the pharmacological actions of the cyanides and of uranium differ from each other much more widely than has been generally believed.

THE FATE OF SACCHAROSE AFTER PARENTERAL INTRODUCTION IN ANIMALS.

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IT is generally assumed that sugars, such as saccharose, lactose, raffinose, etc., which can be inverted in the alimentary tract, but not elsewhere within the organism, are eliminated quantitatively in the urine when they are introduced into the animal body parenterally, *i. e.*, with avoidance of the gastro-intestinal canal.[1] Glycogen formation cannot readily be attained in muscle from parenterally introduced saccharose.[2] This corresponds with the fact that Fischer and Niebel[3] were unable to detect a sucrase in the blood serum of various species (horse, ox, sheep, rat) examined by them. Weinland[4] reported a practically complete urinary elimination of saccharose injected subcutaneously into an *adult* dog; but in puppies the failure to recover the sugar entirely after *repeated* subcutaneous injections of cane sugar was ascribed to the development of a saccharose-inverting enzyme in the blood. The recent physiological literature indicates that all instances of such functional adaptation need to be critically examined before they can be accepted as typical responses on the part of the animal organism. Hohlweg and Voit[5] injected large amounts of saccharose (20 gm.) subcutaneously into rabbits and ordinarily obtained a quantitative return; by raising the temperature of the animals a disappearance of 20 to 25 per cent could be effected.

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[1] The literature is discussed in the paper by MENDEL AND MITCHELL: This journal, 1905, xiv, p. 239. *Cf.* also MAGNUS-LEVY: OPPENHEIMER'S Handbuch der Biochemie, 1909, iv, I, p. 325.

[2] *Cf.* HATCHER and WOLF: Journal of biological chemistry, 1907, iii, p. 25.

[3] FISCHER and NIEBEL: Sitzungsberichte der königlichen preussischen Akademie der Wissenschaften zu Berlin, 1896, p. 73.

[4] WEINLAND: Zeitschrift für Biologie, 1905, xlvii, p. 279.

[5] HOHLWEG and VOIT: Zeitschrift für Biologie, 1908, li, p. 491.

The immediate occasion for a further investigation of this question was given by an experiment reported earlier from this laboratory.[6] A small dog which had received an intraperitoneal injection of 3 gm. of saccharose eliminated 1½ gm. by the kidneys. The urine at no time gave a reduction test with Fehling's solution. This unexpected failure to recover a larger quantity of the carbohydrate under the conditions of the experiment, suggested the inquiry whether there is a specific variation in different animals, whether there may actually be a development of sucrase, whether there are other paths of elimination, or whether the disaccharide is actually utilized to some extent. Fischer and Moore [7] have emphasized the possibility of excretion of sugar through the walls of the alimentary tract after its introduction intravenously in rabbits. Not until our experiments were nearly completed did we become aware of the comparable studies of G. Jappelli and D'Errico.[8] They concluded from experiments on dogs that when cane sugar is introduced directly into the circulation the quantity eliminated in the urine is never equivalent to the amount injected. Both glycosuria and saccharosuria arise, the former desisting first. The blood has no power for inverting saccharose. According to Jappelli and D'Errico saccharose introduced parenterally (intravenously or subcutaneously) is eliminated into the alimentary tract in part through the gastric mucosa, the salivary glands, and, in insignificant degree, the bile. The subsequent fate of this component is obvious.

It was soon found in our own experiments that the inadequacy of the various analytical methods usually employed makes an exact estimation of saccharose in the urine somewhat unsatisfactory. This was complicated by the occasional appearance of a reducing substance in the urine after injection of cane sugar, as well as the levorotatory substance ordinarily present in dog's urine in small amount. The polariscopic method was selected after preliminary trial, as the most satisfactory procedure available, a Schmidt and Haensch triple shadow instrument being used. Tests made with normal dog's urine to which from 1 to 6 per cent of cane sugar was added, indicated minus errors as large as 3.8 per cent when allowance was made for the preexistent levorotation of the secretion. In some of our experiments the rotation was read before and after fermentation of the urine with yeast, in order to correct for the levorotatory substance. Control experiments showed, however, that even this method was not always ideal, the "normal" levorotation frequently being changed quantitatively after fermentation.

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[6] *Cf.* Mendel and Mitchell: This journal, 1905, xiv, p. 246.

[7] Fischer and Moore: This journal, 1907, xix, p. 314.

[8] Jappelli and D'Errico: Atti della R. Accademia Medico-Chirurgica di Napoli, 1903, N. II; 1904, N. II. *Cf.* Maly's Jahresbericht für Thierchemie, 1905, xxxv, p. 79.

At times a reducing substance appeared after parenteral administration of saccharose in the urine of certain — not all — animals. This was especially noteworthy in the dog used in Experiments 1, 2, 3, etc. (see Table I). In addition to the saccharose a notable amount of reducing substance was present in the urine after every injection except the first. Oral administration of cane sugar (Exp. 26, Table I) or injection of invert sugar (Exp. 34, Table I) failed to provoke the elimination of this reducing substance. To what extent the low figures in the calculated elimination of saccharose are attributable to the complication introduced by the diversity of optically active substances present is not apparent. In some trials in which the rotation was determined before and after inversion, the resulting levorotation was far greater than could be accounted for by the saccharose (as determined from the dextrorotation). The reducing substance was therefore probably a levorotatory carbohydrate-levulose, invert sugar, or a mixture of these. The urine itself did not invert cane sugar. This was thoroughly investigated at different temperatures.

The usual routine of our experiments can best be indicated by an illustrative protocol.

Experiment 60. — Bitch, weighing 8 kgm. Urine collected from Feb. 2 to 4 did not reduce Benedict's solution.[9] It showed a levorotation of −1.2° V.[10] On Feb. 4 the animal received a subcutaneous injection (under aseptic conditions) of 50 c.c. of 20.3 per cent saccharose solution = 10.15 gm. or 1.27 gm. per kilo. (The animals were always fed with meat and bone meal, — a diet yielding fæces of dry consistency not likely to contaminate the urine in the metabolism cages).

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[9] *Cf.* Benedict: Journal of biological chemistry, 1909, v, p. 485.

[10] Unless otherwise stated, rotations are here expressed in degrees of the Ventzke scale. 1° Ventzke = 0.3468 angular degree.

Feb. 5, 176 c.c. urine (Sp. g. 1.070) were collected. It reduced the very sensitive Benedict's solution slightly. Rotation, +18.°3 V. = 4.77 per cent of saccharose or 8.4 gm. The urine was diluted and fermented until free from reducing substances. Correcting for the final levorotation thus estimated, the output of saccharose was 8.66 gm., within twenty-three hours. The urine of the next day (220 c.c., sp. g. 1.056) did not reduce Benedict's solution. Rotation, −0.6° V.

Injected — 10.15 gm. saccharose;
Excreted — 8.66 gm. saccharose, or 85 per cent.

A tabular summary of many of our experiments is given below. The dose of saccharose ranged from 1 to 2 gm. per kilogram of body weight and was introduced in sterile solutions of about 20 per cent strength (see Table I).

These data obtained with dogs may be supplemented by a few figures from similar experiments made on cats, at our suggestion, by Mr. W. C. Gibson. The procedure above described for the experiments on dogs was also used in this series. Reduction tests were negative in these cases (see Table II).

The fact that a reducing substance — presumably a sugar — appeared at times in the urine of dogs after injection of saccharose suggests, among other possibilities, that the failure to recover all of the latter may be due to an inversion of the disaccharide in the blood serum by a sucrase, the secretion of which is induced (or the precursor of which is activated) by the sugar injection. An experiment directed to ascertain this gave negative results.

9.30–9.45 A. M. 90 c.c. of 30 per cent saccharose solution were injected into a bitch weighing 13.2 kgm. (*i. e.* about 2 gm. per kgm).

2.00 P. M. Animal anæsthetized; ether and A.C.E.

2.15 P. M. 25 c.c. of blood withdrawn mixed with 10 c.c. 30 per cent saccharose solution, 40 c.c. water and some toluene; placed at 38° (A_1).

25 c.c. of blood withdrawn, boiled and then similarly treated (B_1).

5.00 P. M. Two 25 c.c. portions of blood withdrawn and treated as the first two (A_2 and B_2). A_1, B_1, A_2, and B_2 were kept at 38° for twenty hours. They were then boiled with kaolin and a drop of acetic acid, filtered, cooled, and the rotation determined (100 mm. tube).

TABLE I.

Parenteral Utilization of Saccharose in Dogs.

Number of experiment.	Number of dog.	Weight of animal.	Mode of administration of sugar.	Quantity of sugar introduced.	Time within which elimination took place.	Sugar recovered in urine.			
						Reducing substance.	Saccharose.		
							Calculated without correction for levorotation.	Calculated after correction for levorotation.	Recovery calculated on quantity introduced.
		kgm.		gm.	hrs. min.	gm.[1]	gm.	gm.	per cent
1	1	15.8	subcutaneous	13.9	22	0	7.02	....	(50.5)[2]
2	1	17.0	subcutaneous	18.87	24	1.06+	9.65	....	(51.1)
3	1	16.4	subcutaneous	19.04	22	1.89	5.83	....	(30.6)
5	1	15.8	intraperitoneal	18.99	22 15	1.26	8.23	....	(43.3)
6	1	16.6	subcutaneous	18.26	25 45	1.54	6.82	....	(37.3)
7	1	17.0	intraperitoneal	16.05	29	2.26	7.75	....	(48.3)
10	1	18.2	intraperitoneal	19.46	46	2.58	10.72	....	(55.1)
14	1	19.6	intraperitoneal	9.97	22	1.34	5.37	....	(53.9)
17	1	19.0	intraperitoneal	30.51	23 15	+	17.32	....	(56.8)
21	1	20.6	intraperitoneal	23.03	24 15	+	10.98	....	(47.7)
24	1	19.6	subcutaneous	19.32	49	+	9.84	....	(50.9)
26	1	20.4	oral	42.47	22 10	0	0	....	0
34	1	22.8	intraperitoneal	22.67	23 20	0	0	....	0
[illegible]	[illegible]	11.8	intraperitoneal	Invert sugar 12.20	28 40	trace	8.40		(68.9)

13	Pregnant bitch	13.2	intraperitoneal	13.87	45 45	0	10.31	....	(74.3)
18	13	11.4	intraperitoneal	13.11	29 30	0	8.28	....	(63.2)
20	20	8.0	subcutaneous	17.96	27 45	+	11.18	....	(62.2)
22	20	8.6	subcutaneous	18.74	27 15	0	11.58	....	(61.8)
27	20	8.8	oral	17.31	22	0	0	....	0
35	20	10.0	intraperitoneal	10.05	28 50	....	6.83	8.27	(67.9) 82.3
25	25	4.8	subcutaneous	9.75	17	trace	7.07	....	(72.5)
29	29	13.2	subcutaneous	14.13	22 15	0	10.27	....	(72.7)
31	31	13.0	intraperitoneal	13.09	46 30	0	10.13	....	(77.4)
39	31	13.2	subcutaneous	13.08	45 30	0	9.70	....	(74.2)
36	36	10.0	intraperitoneal	10.05	22 30	0	6.34	6.56	(63.1) 65.3
40	40	12.4	subcutaneous	12.29	70	0	8.28	10.70	(67.4) 87.1
42	40	13.4	intraperitoneal	13.79	46 30	0	10.46	10.94	(75.9) 79.3
41	41	16.2	subcutaneous	16.25	24 15	0	10.68	....	(65.7)
44	41	17.2	intraperitoneal	18.61	49 45	0	12.55	15.09	(67.4) 81.1
48	48	12.2	intraperitoneal	12.50	46 15	0	10.55	12.47	(84.4) 99.8
57[3]	57	10.3	subcutaneous	10.86	71 45	0	7.79	8.45	(71.7) 77.8
59	59	18.0	subcutaneous	19.14	51 50	0	12.18	12.65	(63.6) 66.1
60	60	8.0	subcutaneous	10.15	23	trace	8.40	8.66	(82.8) 85.3

[1] The figures here given are calculated as dextrose by Allihn's method.

[2] The figures in parenthesis are the percentage without correction for levorotation. The figures not in parenthesis are the corrected figures.

[3] After five days' fast.

TABLE II.

PARENTERAL UTILIZATION OF SACCHAROSE IN CATS.

Number.	Weight of animal.	Quantity of sugar injected.	Sugar recovered in urine.		Time within which elimination took place.
	kgm.	gm.	gm.	per cent.	hrs.
5	2.6	3.4	2.7	79	24
13	2.4	2.4	1.3	54	50
16	2.6	3.0	2.8	93	30
14[1]	3.4	3.4	2.8	82	23
15[1]	3.2	3.7	3.2	86	30
2[2]	1.8	4.1	3.6	88	19
4[2]	1.8	2.6	2.2	85	24
6[2]	1.7	2.5	1.1	44	19
8[2]	1.6	4.1	3.6	88	19
9[2]	1.6	4.3	3.6	84	30
10[2]	1.7	4.2	3.8	90	17
11[2]	1.7	5.6	4.9	88	22
12[2]	1.6	4.9	4.6	94	20

[1] Same cat.
[2] Same cat.

Rotation . . A_1 3°.2V A_2 2°.9V
B_1 3°.0V B_2 3°.1V

In a research published since the preceding observation was made, Abderhalden and Brahm[11] have reported comparable experiments in which the serum of dogs was examined for digestive properties after subcutaneous injections of saccharose. In some cases they succeeded in demonstrating a capacity of the serum to transform added carbohydrate soon after an injection of sugar. After repeated injections the serum was often completely inactive; and the authors state that the successful development of the enzyme — never present in the normal serum or in that obtained some time after an injection — depends upon as yet unrecognized factors. At present it is impossible to assign any fixed importance to the mechanism of parenteral sugar utilization here suggested. Further observations are awaited.

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[11] ABDERHALDEN and BRAHM: Zeitschrift für physiologische Chemie, 1910, lxiv, p. 429.

The excretion of saccharose is usually completed within thirty-six hours. — In Experiment 44 (Table I) urine passed two minutes after the (intraperitoneal) injection contained no sugar; in Experiments 17, 24, 9 and 14 (Table I), sugar was present in urines passed five, eight, nine and twenty minutes respectively after its introduction.

In order to ascertain whether, following the experience of Weinland, the apparent utilization could be increased (*i. e.*, the elimination diminished) by some adaptive process, repeated intraperitoneal injections of saccharose were made in a dog at intervals during seven and one-half weeks, approximately 1 gm. per kilogram being introduced each time. As soon as the elimination ceased after each injection,

TABLE III.

Repeated Intraperitoneal Injections of Saccharose in a Dog.

Number.	Weight of dog.	Quantity of sugar injected.	Sugar recovered in urine.		Time within which elimination took place.
	kgm.	gm.	gm.	per cent.	hrs.
1A	18.5	18.6	13.0	70	46
2A	18.6	19.9	12.9	65	72
3A	18.2	20.8	12.95	62	66
4A	18.2	19.2	13.0	68	28
5A	18.4	22.4	14.4	64	65
6A	18.6	23.0	14.6	63	66
7A	18.8	23.1	...	..	..
8A	18.4	20.4	13.8	68	43
9A	18.1	18.6	18.0	97	40
10A	18.8	19.8	...	..	..
11A	18.6	20.9	14.1	67	45
12A	19.0	18.7	13.1	70	48

TABLE IV.

PARENTERAL UTILIZATION OF SACCHAROSE IN PUPPIES.

Number.	Age of animal.	Weight of animal.	Mode of administration of sugar.	Quantity of sugar introduced.	Time within which elimination took place.	Sugar recovered in urine.		
						Reducing substance.	Saccharose. Calculated without correction for levorotation.	Recovery calculated on quantity introduced.
	days.	kgm.		gm.	hrs. min.	gm.	total gm.	per cent.
30	43	1.8	intraperitoneal	1.93	27 15	0	1.53	(79.3)
38[1]	56	2.6	intraperitoneal	2.61	29 10	0	2.00	(76.6)
32	46	2.0	intraperitoneal	2.01	21 40	0	1.82	(90.5)
37[2]	56	2.4	intraperitoneal	2.41	29 15	0	1.98	(82.2)

[1] Same animal as 30.

[2] Same animal as 32.

TABLE V.

PARENTERAL UTILIZATION OF SACCHAROSE IN DOGS AFTER ALCOHOL INTOXICATION.

Number	Weight of animal.	Mode of administration of sugar.	Quantity of sugar introduced.	Time within which elimination took place.		Sugar recovered in urine.			
						Reducing substance.	Saccharose. Calculated without correction for levorotation.	Saccharose. Calculated after correction for levorotation after fermentation.	Total recovery calculated on quantity introduced.
	kgm.		gm.	hrs.	min.		gm.	gm.	per cent.
33[1]	9.8	intraperitoneal	9.87	22	30	0	5.52	....	(55.9)
35[2]	10.0	intraperitoneal	10.05	28	50	....	6.83	8.27	(67.9) 82.3
48[3]	12.2	intraperitoneal	12.50	46	15	0	10.55	12.47	(84.4) 99.7
51[4]	11.4	intraperitoneal	10.37	48	30	++	6.54	10.25	(63.1) 98.8
54[5]	11.4	subcutaneous	11.64	23	10	+	7.30	9.52	(62.7) 81.8
56[6]	11.6	subcutaneous	11.70	41		+	8.25	8.42	(70.5) 72.0

[1] Ninety-four c.c. of 95 per cent alcohol and 200 c.c. water administered orally before the injection; 19 c.c. alcohol and 40 c.c. water after the injection (= 11½ c.c. 95 per cent alcohol per kgm.)

[2] Same animal as 33, no alcohol.

[3] No alcohol.

[4] Same animal as 48, 68 c.c. 95 per cent alcohol and 137 c.c. water administered orally before injection (= 6 c.c. 95 per cent alcohol per kgm.)

[5] Same animal as 48, alcohol as in 51.

[6] Same animal as 48, no alcohol.

the dog was given freedom for a day or two before the experiment was repeated. Some of the analytical results are recorded in tabular form.[12] No improvement in utilization was noted (see Table III). The *repeated injections* in the same cat (Exps. 2–12, Table II) point in the same direction. *Fasting* did not materially increase the ability to utilize the sugar parenterally (*cf.* Exp. 57, Table I); nor was the extent of elimination specially altered in a *pregnant* animal (*cf.* Exps. 13 and 18, Table I). The influence of *age* was studied in several puppies of the same litter[13] (see Table IV).

In view of the assumed *influence of alcohol* on the metabolism of carbohydrates, a few experiments on sugar utilization were tried after administering large doses of alcohol. The data are summarized in Table V.

Finally it will be noted that in a few experiments (Exps. 20, 22, and 25, Table I) where sugar was injected in doses of 2 gm. per kilogram of body weight the outcome as regards utilization was not noticeably modified.

SUMMARY.

When saccharose is introduced parenterally (intraperitoneally or subcutaneously) into dogs and cats in doses of 1 or 2 gm. per kilogram of body weight, it is not completely recovered in the urine. The quantity excreted amounts, as a rule, to more than 65 per cent of that introduced. Under these conditions a reducing substance is sometimes excreted. It is presumably levorotatory, but its exact nature is as yet undetermined. Technical difficulties make the quantitative data somewhat inexact.

The excretion of parenterally introduced saccharose begins within a few minutes and is usually completed within thirty-six hours.

The influence of various conditions: age, fasting, pregnancy, alcohol intoxication, repeated injections, has been studied. Although the absence of sucrase from the blood, even during the period of sugar absorption, has again been demonstrated in our experiments, the observations of Abderhalden and Brahm leave the possibility of a parenteral digestion of saccharose in the blood to be reckoned with in accounting for the disappearance of part of the carbohydrate.

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[12] Most of the trials were made by Mr. W. C. Gibson.

[13] *Cf.* Doyon and Dufourt: Journal de physiologie, 1901, iii, p. 703, on the influence of such conditions upon the utilization of dextrose given intravenously.

THE CONCENTRATION OF AMMONIA IN THE BLOOD OF DOGS AND CATS NECESSARY TO PRODUCE AMMONIA TETANY.

By CLARA JACOBSON.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

THE work of determining the concentration of ammonia in the blood necessary to produce ammonia tetany was undertaken in view of its relationship to the increased blood ammonia in animals in parathyroid tetany. In a recent paper[1] this ammonia increase was shown to be apparently associated with an impaired liver activity. These factors — increased ammonia concentration and liver insufficiency — as well as the marked similarity of the symptoms following parathyroidectomy to those of ammonia intoxication or of excessive meat feeding after Eck fistula, all suggest that ammonia may be the cause of the parathyroid tetany. Some ammonia is always present in the blood. Its concentration is normally regulated by the conversion of its excess into urea and by elimination. Thus the relatively large quantity produced in exogenous and endogenous metabolism, particularly the former, does not, in a normal animal at least, materially change the concentration in the general systemic circulation. These processes tend to explain the fact that after injection of a quantity of ammonia sufficient to produce symptoms of poisoning, recovery may be very rapid — the tetany or extreme manifestations often totally disappearing within a few minutes while the associated depression may continue for an hour or more. The latter condition is probably due in part to a primary action of ammonia, in part to secondary effects arising from the general constitutional derangement and exhaustion. The primary symptoms at a given time are undoubtedly dependent upon the increased concentration of ammonia in the circulating blood and in the tissues and lymph. The direct analysis of the blood during an ammonia convulsion will therefore afford another basis of comparison and may tend either to support or to disprove the theory that the parathyroid tetany is due, at least in part, to the ammonia.

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[1] Carlson and Jacobson: This journal, 1910, xxv, p. 403.

METHODS.

A solution of 5 to 10 per cent ammonia carbonate was injected intravenously until just sufficient to cause the appearance of acute symptoms of ammonia poisoning. In dogs these injections were made into the saphenous vein, and in cats into the external jugular previously isolated under ether anæsthesia. In some cases, especially among the cats, the convulsions were accompanied by respiratory standstill and artificial respiration was necessary to prevent death from asphyxia. The blood samples were collected four or five minutes after the injections, while the animals were still in tetany or tremors. Thus the ammonia was allowed to circulate freely, to mix well with the blood, and possibly establish a condition of temporary equilibrium in the tissues and the lymphs. Variations in the results are to be expected because of the individual differences in the severity of symptoms shown; because of variations in the time elapsing between the injection and the drawing of the blood, and owing to variations in the actual time required for the drawing of sufficient blood for analysis. The first mentioned factor is of relatively slight importance here, since it is also present among the thyroidectomized animals. The ammonia analyses were made according to the method of Folin.

RESULTS.

Blood samples from dogs, normal (including one case in which the pancreas had been removed two weeks previously) and in parathyroid tetany, were analyzed. These results are tabulated with those of the ammonia injections (see Tables I and II).

The symptoms following the injection of ammonia are very similar to those of thyroidectomy. It is not necessary to go very much into detail in their description, as this has already been done by a number

TABLE I.

Ammonia Content in Mgm. per 100 c.c. of Blood of Dogs.

No.	Normal.	In parathyroid tetany.	In ammonia tetany.
1	1.632	2.73	2.8
2	1.496	2.79	2.1
3	1.49	2.86	3.7
4	1.22	2.08 (2.4[2])	1.75
5	1.36	2.88	2.45
6	1.43[1]	3.00	...
7	...	1.82[3] 2.275	...

[1] Second week pancreatic diabetes.

[2] Second blood sample drawn twenty-four hours after the first.

[3] The animal was only slightly depressed when the first sample of blood was drawn but showed profuse salivation, dyspnœa, and tetany when the second sample was drawn six hours later.

TABLE II.

Ammonia Content in Mgm. per 100 c.c. of Blood of Cats.

No.	Normal.	In parathyroid tetany.[1]	In ammonia tetany.
1	1.530	2.635	3.445
2	1.445	2.176	3.640
3	1.900	2.516	1.885
4	1.445	3.230	2.405
5	1.530	2.516	2.47
6	...	2.125	3.18
7	...	...	2.34

[1] Quoted from Carlson and Jacobson: This journal, 1910, xxv, 410, Table I.

of investigators. There is salivation with an irregularity of respiration, usually rapid and shallow; sometimes slow, deep and labored. In some of the cases where the injections were slow, a primary depression and even somnolence was noted (two of the dogs). Then convulsions set in. In cats, especially, the symptoms appeared suddenly, being marked by tetany, muscular tremors in varying intensity, intermittent rigidity and respiratory standstill. Tetany usually predominated, but in one instance at least tremors without tetany were noted. The rigidity with respiratory standstill observed in four cats was similar to that observed in several thyroidectomized cats. During and following this convulsive phase a very marked hyperexcitability was present. In dogs the symptoms were very similar but apparently less acute as compared with those of cats.

Thus out of the six dogs, only two showed respiratory standstill requiring artificial respiration, though in two others there was intermittent rigid extension of the limbs of short duration. Tetany, too, was generally more marked than the muscular tremors, but one instance was noted in which tremors were very strong but unaccompanied by tetany. In recovery from the injections most of the dogs showed extreme depression and weakness.

DISCUSSION OF RESULTS.

The concentration of ammonia in the blood of cats and dogs in ammonia tetany is found to be practically equal to that in parathyroid tetany. This does not constitute a conclusive proof of the ammonia intoxication theory because there are many possible complications. The susceptibility to the ammonia depends probably in the main on two factors, namely, the form of the ammonia and the degree of excitability of the central nervous system. In the thyroidectomized animals there may be other conditions that modify the excitability, such as the supposed changes in calcium and magnesium salts. We do not know in what form the ammonia is present in the blood of these animals nor that the ammonia is the only protein derivative directly involved. The work of Berkeley and Beebe[2] renders it probable that other products of protein metabolism, such for instance as the purin bases, also contribute to the effects. The constant presence of an excess of ammonia in the blood may augment the excitability or tend to establish a tolerance. Thus the condition of the animal and the form of the ammonia compound enter in as two variable factors. However, the fact that the symptoms produced by the injection of ammonia are so similar to those following the removal of the thyroid-parathyroid and the further fact that the concentration of ammonia present in thyro-parathyroid tetany is sufficient to cause tetany in normal animals, give support to the ammonia intoxication theory.

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[2] BERKELEY and BEEBE: Journal of medical research, 1909, xx, p. 149.

Little can be added to what has already been said regarding the cause of this increased ammonia. According to our results the liver is depressed especially as to its power of destroying ammonia. There appears to be little or no histological change associated with this depression. Delitala[3] observed varying degrees of fatty degeneration in the livers of dogs in parathyroid tetany; but there was no constant relationship between severity of the symptom and the extent of degeneration. In fact in some animals showing most severe symptoms, the liver showed little change. So far as we have observed, the livers of animals in parathyroid tetany present no gross abnormalities. Dr. H. G. Wells has kindly made microscopical examinations of the livers of three such dogs and one fox and found by sudan III stain a central fatty degeneration, one half or less of the radius of the lobules being involved. In one the cytoplasm of the cells in the centre of the lobules took a lighter stain with eosin than normal. Livers in which fatty changes exceeding those above described have been produced by poisoning as with phosphorus show apparently no depression of the ammonia-destroying power. It seems therefore impossible as yet to demonstrate micro-chemical changes in liver tissue sufficient to account for the decreased activity.

CONCLUSIONS.

The concentration of ammonia in the blood of cats and dogs in ammonia tetany is practically equal to that in parathyroid tetany.

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[3] DELITALA: Archives italiennes de biologie, 1908, xlix, 109.

This supports the view that the increased ammonia in the blood of parathyroidectomized animals is directly responsible for the tetany and the depression symptoms.

This work was done under the direction of Dr. Anton J. Carlson, and I wish to express my thanks for his assistance, criticism, and encouragement.

THE RATE OF HEALING OF WOUNDS IN DENERVATED SKIN AREAS AND ITS BEARING ON THE THEORY OF TROPHIC NERVES.[1]

By CLARA JACOBSON.

[From the Hull Physiological Laboratory of the University of Chicago.]

THE maintenance of life processes in plants and in the lower animals seems to be largely a phenomenon of chemical co-ordination. But with the increased development of the nervous system there appears the possibility of a specific or direct nervous control of the metabolism and the growth of individual cells of the tissues. If such is the case, the dependence of the tissue cells on these "trophic" nervous impulses ought to be greater in the higher than in the lower animal phyla. But even in the highest vertebrates at least one tissue, namely, the blood, or rather the formed elements of the blood, is not directly influenced by any nervous impulses but is maintained by chemical co-ordination. The following experiments were made with the view of testing out this hypothesis, suggested by Dr. Carlson to determine at what stage of phylogenetic development trophic nervous impulses could be demonstrated. Thus pigeons were first used and later similar experiments made on dogs. The presence of an interrelationship between the growth and number of motor-nerve fibres and the growth of the corresponding musculature, embryologically,[2] does not give definite evidence in favor of the trophic theory. The fact that these processes are apparently equally dependent upon one another suggests a chemical co-ordination.

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[1] The following partial Bibliography may be of service: GAULE: Centralblatt für Physiologie, 1891, v, p. 409; ECKHART: *Ibid.*, 1892, vi, p. 328; JOSEPH: Archiv für pathologische Anatomie und Physiologie, 1887, cvii, p. 119; KÖSTER: Zur Physiologie der spinal Ganglien und der tropischer Nerven sowie zur Pathologenese der Tabes Dorsalis, Leipzic, 1907; BIKELES and JASHINSKI: Centralblatt für Physiologie, 1898, xii, p. 345; TRENDELENBURG: Neurologischer Centralblatt, 1906, xxv, p. 386; ARNDT: Archiv für Physiologie, 1891, p. 54; DUNDURFI: Centralblatt für die allgemeine Pathologie und pathologische Anatomie, 1894, v, p. 509; GOLTZ and EWALD: Archiv für die gesammte Physiologie, 1896, lxiii, p. 362; GAULE: Centralblatt für Physiologie, 1891, v, p. 450; HEAD and SHERREN: Brain, 1905, xxviii, p. 116; RIVERS and HEAD: Brain, 1908, xxxi, p. 323; TROTTER and DAVIES: Journal of physiology, 1909, xxxviii, p. 134.

METHODS.

Pigeons were put under ether anæsthesia, and an incision made in the mid-dorsal line in the lumbar region, a line drawn across the tips of the trochanters being used as a guide. Here the pigeon's anatomy is peculiarly well adapted to the operation in that there are no muscles and very little fascia separating the skin from the bone in the rhomboidal space about 3 cm. long by 1½ cm. at the widest, and in that the bone is soft and spongy and can be easily removed leaving a smooth opening to the cord. The cord and right nerve roots were exposed for a distance of about 15 mm. In the first series of experiments dorsal roots alone were cut. Increased excitability was frequently noticed, as well as some muscular twitchings probably due to traction on the cord. There was often considerable hemorrhage from the rupture of meningeal vessels, but in no case did this result seriously or even produce any noticeable effect. After recovery from anæsthesia there was apparently no loss of voluntary control of the muscles. The insensitive areas were then determined, uniform wounds (cuts) were made in these and the corresponding areas of the opposite side and the rate of healing compared. At first all due aseptic precautions were attempted in the making of these wounds, but were later found unnecessary as pigeons are very resistant to ordinary infection. In some cases secondary wounds were made subsequent to the healing of previous ones in an attempt to see if the time element entered in.

After being under close observation for about a month, these birds were again anæsthetized, the cord exposed and the ventral nerve roots of the same area sectioned. In other birds both dorsal and ventral roots were sectioned simultaneously. Paralyses of the foot and leg muscles were practically complete, slight control of the position of the limb being maintained, however, in the upper thigh and trunk muscles. Wounds similar to the previous ones were made, and, repeatedly, observations extending over a period of six months.

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[2] SHOREY: Journal of experimental zoölogy, 1909, vii, p. 25.

RESULTS.

In the first series, section of the dorsal roots, there were seven cases in each of which wounds healed just as rapidly on the right leg as on the left.

In the second series, section of the ventral roots, ten pigeons were under observation, wounds being made immediately after the operation, then again eight to fifteen days later and on four birds six months later. In no case was there any detectable difference in the rate of healing of the wounds. There was no difference noticeable in the general appearance of the denervated limb, such, for instance, as described by Trendelenburg.

Drs. Carlson and Werelius[3] in continuation of this subject made observations upon dogs in three of which they severed the dorsal cutaneous rami of three contiguous spinal nerves, and in three others the posterior roots of three contiguous spinal nerves. The areas of anæsthesia were mapped out and shaved and similar wounds made under strictly aseptic conditions on the completely insensitive area and on the corresponding area of the normal side. Bandages were applied to keep the conditions aseptic and uniform on the two sides. In none of these cases were any differences noticeable in the rate of healing or in the growth of the hair.

DISCUSSION OF RESULTS.

These results indicate that in the pigeon and the dog the section of nerves, afferent, efferent or both, to a part is not followed by a diminished rate of healing of lesions made either immediately after the operation or at any time during a period of six months. Trophic changes are most frequently associated with injury to so-called sensory nerves or nerve roots, but there are some, as has been previously stated, who have brought forth evidence to show that such changes may be related to the sympathetic nervous system, and there may be still others who consider the atrophy of muscles after section of their

[3] Personal communication.

motor nerves as not wholly due to disuse. In the second case, the disturbances are probably secondary to vaso-motor influences. In the last, the possibility of preventing atrophy of muscles while the nerves are regenerating by direct artificial stimulation and the absence of specific nerve endings other than motor nerve endings seem to argue against the trophic theory.

The variation in the results of different observers may be largely due to individual variations in animals, just as in other lines of research; for instance, the cases in which a heavy meat diet after Eck fistula does not result in poisoning symptoms, the opposite being the general rule, and the failure of tetany and death in some dogs after apparently complete thyroidectomy. However, in these experiments, negative evidence is of more value than positive, in fact may even be regarded as positive since the absence of disturbances in metabolism and growth in denervated areas indicates an ability to dispense with trophic impulses if such are normally present, or, in fact, renders the theory of trophic nervous impulses unnecessary. The demonstration of changes may be proof toward the existence of trophic nerves, but on the other hand there are invariably so many contributing causes as in case of ulcers in tabes or of keratitis following section of the Gasserian ganglion. Vaso-motor effects may also enter in as results of increased vaso-motor reflexes, as may be the case in infection of the spinal cord or of the dorsal root ganglia, or the cutting off of these reflexes in infection or in section of nerves. The apparently increased susceptibility to infection in peripheral areas following the section of nerves or especially following the transsection of the cord as in Goltz's animals seems to give support to the trophic theory, but it may be due to the anæsthesia with resulting lack of attention on the part of the animal to slight or serious injuries, and possibly some disturbance of vaso-motor reflexes. Vaso-motor paralysis in which dilatation is not as great as possible under stimulation, may be a factor in causing the diminished growth of feathers observed by Trendelenburg. In Head's case, the facts that the source of the lesion was so severe, freezing by ethyl chloride, and that such wounds are difficult to heal under more normal conditions when the sebaceous and sudoriferous glands are active, the skin elastic, and normal vaso-motor and other reflexes present, seem to argue against the view that the slow rate of healing was due to lack of trophic impulses. The final healing of the wound may have been a coincidence, favored possibly by the return of vaso-motor reflexes with the return of protopathic sensitivity.

Some tissues will live and grow even though their nerve supply is cut off. Organs such as the thyroids, hypophysis, pancreas, adrenals, mammary glands, ovaries, testes, or kidneys may be successfully transplanted in part, and growth and function continue. Carrel and Guthrie[4] even obtained primary union in transplanted legs. One may suppose that this is due to the ingrowth of the nerves to the part, but such regeneration would probably be comparatively slow — taking a longer time than necessary for the complete re-establishment of the function of the organs. The presence of peripheral ganglion cells within the organs may also be suggested in answer to their growth after having been transplanted or denervated, but there is one instance at least wherein the presence of such a peripheral ganglion has not the power to prevent atrophy of the organ after section of its nerve, namely, in the sub-maxillary gland. This is also clearly not a case of atrophy from disuse, for the gland continues to secrete. It is probable that these gland cells not being adapted to continued secretion suffer exhaustion. The compensatory hypertrophy of organs after the removal of the collateral organ is probably evidence of chemically stimulated growth. There are other instances which indicate that in general the maintenance of life and the growth of tissues belong in the class of phenomena which are chemical or of the hormone type rather than nervous.

CONCLUSIONS.

1. The results of the experiments reported indicate no diminution in the rate of healing of wounds in a denervated (sensory or sensory and motor) skin area as compared directly with that in a normal area.

2. The variations in the results obtained by different investigators may be explained on the grounds of individual variation among animals.

3. It seems that so-called trophic disturbances may be due to vaso-motor changes with increased susceptibility to infection or to the loss of protective reflexes from loss of sensitivity to injurious agents.

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[4] CARREL and GUTHRIE: Science, 1906, xxiii, p. 393.

I undertook this work under the direction of Dr. A. J. Carlson, and wish to express my appreciation for his constant interest and encouragement and for the many valuable suggestions he has offered during the progress of the work.

ADDENDUM.

The above conclusions respecting trophic nerve fibres or nervous impulses coincide in part with the position taken by Tschermak, whose critical review of the literature on trophic and tonic innervations has just appeared (Folio Neuro-Biologiae, 1910, iii, p. 676). Tschermak admits that there is no evidence for the existence of separate or specific trophic nerve fibres, but considers it highly probable that motor and sensory as well as afferent nerve fibres convey trophic impulses as an accessory function ("Teilfunktion"). I fail to see the possibility of this distinction between motor-sensory-tonic and trophic impulses in the same axis cylinder on the basis of our present knowledge, or the advisability of retaining the term trophic for this conception. It seems to be true, in general, that moderate amounts of the special activity of organs are favorable to their metabolism and growth. The impaired metabolism and consequent atrophy in muscle, glands, and nerve centres following lesion of motor, secretory, and afferent pathways respectively can therefore be wholly accounted for as a direct result of the cessation of the special organ activity. Tschermak's hypothesis involving us in the difficulty of conduction of qualitatively different impulses along the same axis cylinder, should be the last, not the first resort. It would seem, then, that all the changes in muscle, gland and nerve centres following severance of their nervous connections are a direct result of disuse or altered special activity, and do not call for even the modified trophic hypotheses of Tschermak. But the case of epithelial surfaces such as the skin and the cornea does not appear to be so simple, and most of the evidence in support of the trophic nerve theory is derived from these very tissues (skin, hair, feathers, etc.). The apparent trophic influence of the central nervous system on the skin cannot be an indirect effect due to the secretory nervous impulses to sweat gland and sebaceous gland or motor nervous impulses to the smooth musculatures related to the skin, hair and feathers. Impaired activity of the sebaceous glands may lead to changed appearance of the skin surface, but it is difficult to see how it could result in altered growth in the deeper epithelial strata. Section of a cutaneous nerve results in temporary active hyperemia, but the increased flow and pressure in the blood capillaries ought not to depress the metabolism of the skin unless accompanied by edema. Evidence is accumulating to the effect that in the exchange between the blood and the tissue cells the capillary cells act not only (if at all) as simple membranes for filtration, transfusion and osmosis, but also as secreting membranes. To what extent this secretory activity of the capillary cells is governed directly by nervous impulses is not known. The capillary endothelium in many parts of the body, at least, is supplied with nerves and nerve endings, but their physiology is a matter of conjecture. Assuming that some of these capillary nerve fibres are efferent or secretory, lesion of these nerves would probably result in changed activity of the capillary cells, and this in turn would result in altered conditions of the tissue cells. It would then seem that the theories of trophic nerve fibres and trophic impulses in nerve fibres having other functions are equally superfluous. The changes in muscle, gland and nerve cells in consequence of lesion of or altered activity of conduction paths are probably the result of disuse or altered special organ activity. And the so-called trophic changes in the skin and epidermal structure can be accounted for by altered activity of the capillary cells in consequence of the hyperemia, increased pressure and possibly interference with secretory fibres.

A. J. C.

THE SEPARATION AND ESTIMATION OF ASPARTIC AND GLUTAMINIC ACIDS.[1]

By THOMAS B. OSBORNE AND L. M. LIDDLE.

[*From the Laboratory of the Connecticut Agricultural Experiment Station.*]

IN analyzing the mixture of amino-acids which result from protein hydrolysis it is important to take advantage of every means by which the present methods can be improved.

One opportunity for facilitating the separation of some of the amino-acids is presented by the fact that the dibasic aspartic and glutaminic acids react distinctly acid towards litmus but in the form of their very soluble acid sodium salts are neutral thereto. In consequence of this acidity these two amino-acids may combine with the amino groups of such other amino-acids as may be present, and thereby produce mixtures from which the several components can be separated with great difficulty if at all.

In addition to the opportunity thus offered for improving the separation of mixtures of amino-acids it seemed that it would also be possible, not only to detect the presence of dibasic amino-acids in mixtures, but also, if only one of them was known to be present, to measure its amount.

In conducting a protein analysis by Fischer's method it is possible in some cases to obtain glutaminic acid hydrochloride contaminated with leucine hydrochloride, and it has been found that, when this happens, it is practically impossible to separate these amino-acids by direct crystallization. As an illustration of this the following experiment may be cited:

Five grams of leucine and 5 gm. of glutaminic acid were dissolved in 50 c.c. of dilute hydrochloric acid, the solution saturated with hydrochloric acid gas, and placed on ice for some time. The hydro-

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[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

chlorides which separated were sucked out, washed with ice-cold alcoholic hydrochloric acid, dissolved in 30 c.c. of water, and again precipitated by saturating with hydrochloric acid gas. After cooling on ice for some time the crystalline separation was filtered out and washed with cold, concentrated alcoholic hydrochloric acid. The substance was then dissolved in water, chlorine removed with silver carbonate, and the solution evaporated to dryness. The residue, which weighed 4.88 gm., was then dissolved in water and made neutral to litmus with 54.8 c.c. of N/2 sodium hydroxide solution. This quantity of alkali is equivalent to only 4.03 gm. of glutaminic acid, thus indicating the presence of 0.85 gm., or nearly 18 per cent, of leucine in the residue which had been twice precipitated as hydrochloride.

The filtrate from the first precipitation of the hydrochlorides was freed from chlorine by evaporation and treatment with silver carbonate, evaporated to dryness, and the residue dried to constant weight at 110°. This residue, which weighed 2.47 gm., required 4.7 c.c. N/2 sodium hydroxide solution for neutralization to litmus, indicating the presence of 0.34 gm. of glutaminic acid.

The filtrate from the second precipitation as hydrochloride, when similarly treated, yielded a residue weighing 1.94 gm., which required 5.2 c.c. of the alkali to make its solution neutral to litmus, which corresponds to the presence of 0.38 gm. of glutaminic acid.

The fact that the first filtrate from the hydrochlorides contained only 2.47 gm. of substance shows plainly how readily leucine may be precipitated together with glutaminic acid as the hydrochloride, and the results as a whole show how difficult it is to separate these two amino-acids under such conditions.

We have already shown that in attempting to separate a mixture of glutaminic acid and leucine hydrochlorides only a part of the glutaminic acid hydrochloride could be separated as such, for after concentrating and cooling the solution from which much relatively pure glutaminic acid hydrochloride had separated, the remainder crystallized together with leucine hydrochloride. After removing all of the hydrochloric acid the mixture of free glutaminic acid and leucine still failed to yield any satisfactory products when subjected to direct crystallization.

When, however, the solution was made neutral to litmus, the leucine crystallized out readily, and from the mother liquors it was then possible to separate a further considerable quantity of glutaminic acid hydrochloride.

In analyzing proteins according to Fischer's ester method it is difficult to obtain a sharp separation of leucine and aspartic acid esters by distillation at low pressures. Unless the distillation is so conducted as to effect a reasonably complete separation, the leucine fraction may be sufficiently contaminated with aspartic ester to make the separation of its constituents extremely difficult. On the other hand, if the leucine ester contaminates the upper fraction, its presence greatly interferes with the isolation of phenylalanine in a pure condition, and furthermore the leucine itself cannot be separated and is consequently lost from the reckoning.

If it were possible to separate leucine from aspartic acid or to estimate quantitatively the one in the presence of the other, it would simplify the analysis and add distinctly to its accuracy, for a small fraction could then be taken out when distilling the esters, which would consist essentially of leucine and aspartic esters. By keeping this small fraction separate all danger of contaminating the lower leucine fraction with aspartic acid or the upper aspartic acid fraction with leucine can be avoided. We have therefore tried the following experiments in the hope of finding some means of meeting the difficulties heretofore encountered in separating these two amino-acids:

In order to learn with what accuracy aspartic acid can be estimated by titration, both alone and in the presence of leucine, we prepared a 1/20 N solution of aspartic acid and a 1 per cent solution of leucine, and neutralized various volumes of each with a 1/2 N solution of sodium hydroxide to a distinct blue reaction with delicate neutral litmus paper. The results are given in the following table:

	$\frac{N}{20}$ Aspartic Acid Solution. c.c.	1% Leucine Solution. c.c.	$\frac{N}{2}$ NaOH	
			Used. c.c.	Calculated. c.c.
I	100	0	9.95	10
II	150	0	14.90	15
III	0	50	0.05	0
IV	100	50	10.00	10
V	100	100	10.10	10
VI	100	200	10.00	10
VII	50	3 gm.	5.1	5

As the separation of leucine and aspartic acid by fractional crystallization presents especial difficulties, we prepared a solution containing 3.325 gm. of aspartic acid and 7 gm. of leucine. This was made neutral to litmus with 50.1 c.c. of one-half normal sodium hydroxide solution. By successive concentration and crystallization from dilute alcohol, four crops of pure leucine were obtained, weighing 3.54, 0.96, 0.84, and 0.49 gm. respectively. Another crop weighing 0.53 gm. was not quite pure. The total leucine thus crystallizing out directly weighed 6.36 gm., equal to 90.8 per cent of that present in the solution.

The filtrate from the leucine was then evaporated to a small volume and 47 c.c. of N/2 HCl (a little less than the calculated amount required to neutralize the alkali previously added to the solution) was added. Four successive crops of aspartic acid were then separated, weighing respectively 1.72, 0.45, 0.28, and 0.1 gm., making a total of 2.55 gm., or 76.8 per cent of the 3.32 gm. in the original solution.

In a second experiment with a solution containing 15 gm. of leucine and 7 gm. of aspartic acid we recovered 14.04 gm. of leucine and 5.54 gm. of aspartic acid, or 93 and 79 per cent respectively. Although these results leave much to be desired in point of accuracy, they approximate a complete separation more nearly than has heretofore been possible.

As an illustration of the behavior of a mixture of leucine and aspartic acid when subjected to direct crystallization, the following may be cited: A mixture of 4 gm. of leucine and 2 gm. of aspartic acid was dissolved in hot water, a little alcohol added and allowed to stand over night. The first crop of crystals, which weighed 1.4 gm., required 11.1 c.c. N/2 sodium hydroxide solution for neutralization. This corresponds to 0.74 gm. of aspartic acid and 0.69 gm. of leucine.

The filtrate, concentrated to incipient crystallization, yielded a second crop, which weighed 1.42 gm. This neutralized 1.1 c.c. of the alkali, corresponding to 0.07 gm. of aspartic acid and 1.35 gm. of leucine.

The mother liquor from the second crop of crystals neutralized 18.2 c.c. of the alkali, indicating the presence of 1.15 gm. of aspartic acid and, by difference, 2.03 gm. of leucine.

From this it is clear that these two amino-acids cannot be thus separated, for although at times a crop of nearly pure leucine or aspartic acid may crystallize out, a complete separation of either one or the other cannot be obtained.

In order to apply this method under conditions similar to those of a protein analysis we esterified a mixture of 30 gm. of leucine and 15 gm. of aspartic acid, extracted the esters with ether according to Fischer's directions, and then distilled them at about 35 mm. pressure. The leucine ester began to distil rapidly at 107° (vapors), the temperature gradually rising to 142°. Most of the ester which distilled between these points came over below 120°, above which very little passed over. As the distillation began to increase in rate at 142°, the first fraction was collected at this point.

In order to saponify any aspartic ester which the distillate might contain it was boiled with baryta solution according to the method usually applied to the higher boiling fractions of the esters. After removing the baryta from the first fraction the solution was concentrated to about 500 c.c. and titrated with N/2 sodium hydroxide solution to a distinct blue reaction with neutral litmus paper. This required 16.7 c.c., corresponding to 1.11 gm. of aspartic acid.

By fractional crystallization from dilute alcohol 19.4 gm. of leucine were obtained. The mother liquor was then evaporated to about 50 c.c. and 16.5 c.c. N/2 hydrochloric acid solution added. After standing some time 0.65 gm. of pure aspartic acid separated.

The mother liquor when evaporated to dryness left a residue weighing 1.85 gm. Since 0.48 gm. of sodium chloride was formed from the added alkali and acid, the presence of 1.37 gm. of leucine and aspartic acid is indicated. As the amount of alkali required to neutralize the original solution corresponded to 1.11 gm. of aspartic acid, of which 0.65 gm. was isolated as such, it can be assumed that the residue contained 0.44 gm. of aspartic acid and 0.91 gm. of leucine.

The undistilled esters were saponified with baryta solution in the usual way, and after removing baryta the solution was concentrated and cooled, whereupon 7.46 gm. of aspartic acid separated in well-developed crystals. The mother liquor on evaporation left a residue weighing 1.38 gm. Since this required 11.2 c.c. N/2 sodium hydroxide for neutralization to litmus, it can be assumed to contain 0.74 gm. of aspartic acid and 0.64 gm. of leucine.

The results of these determinations are given in the following table:

Estimated by	Leucine. gm.	Aspartic acid. gm.	
Weighing	19.4	0.65	Fraction I
Titration	0.9	0.46	
Weighing		7.46	Fraction II
Titration	0.64	0.74	
Recovered	20.94 = 69.8%	9.31 = 62%	of amount taken.

These figures do not represent the total amount of these amino-acids that can be recovered after esterifying, for a considerable accidental loss occurred on liberating the esters with sodium hydroxide solution. The ratio of the recovered aspartic acid to the recovered leucine is nearly the same as that in the original mixture.

From these figures it would seem possible to effect a satisfactory separation of leucine and aspartic acid by taking out a small fraction of the esters after most of the leucine had distilled over and before the aspartic ester begins to distil freely and then treating this separately in the above described manner.

CONGENITAL THYROIDISM: AN EXPERIMENTAL STUDY OF THE THYROID IN RELATION TO OTHER ORGANS OF INTERNAL SECRETION.[1]

By R. G. HOSKINS.

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THE following paper is a report of a part of a series of researches that have been undertaken in an attempt to throw some further light on the inter-relations of the endosecretory organs.[2] They have been purely morphological studies, based upon the supposition that any influence acting upon one of a congeries of related organs would tend to cause increased or decreased activity in each of the others, which would in turn tend to cause a corresponding hypertrophy or atrophy. Various methods have been used in the studies, but in this report attention will be confined to a single series of experiments in which pregnant guinea pigs were subjected to hyperthyroidism with the intent of influencing the gland weights of their offspring. The effects of the procedure were studied in the adrenals, the hypophysis, the ovaries, the pancreas, the testes, the thymus, and the thyroid. The weights of the spleen, kidneys, and liver were also determined to serve as controls on other glands that might be specifically affected by the experimental procedure.

Preliminary to the investigations proper, it was necessary to determine norms for the different glands.

New-born animals were lightly etherized and, in order to eliminate variability in gland weights due to differing blood content, were bled to death from the aorta. Then the various organs under investigation were carefully dissected out and weighed. With the aid of a Zeiss binocular dissecting microscope, it was found possible to secure each

[1] Published in abstract in Proceedings of the American Physiological Society, This journal, 1910, xxv, p. xii. The work was aided by a grant from the "Bowditch Fund for the Promotion of Physiological Research."

[2] *I. e.*, organs of internal secretion.

gland with considerable ease, entirely free from adventitious tissue. Later it was found possible to dispense with the microscope by removing the glands directly to a piece of smooth dry cork and there trimming off the extraneous tissue. This can most effectively be done by rolling the moist organ over the dry cork; the loose tags which adhere to the cork can readily be seen, and trimmed off with a sharp scalpel. A subsequent inspection with the microscope has shown that the method is entirely satisfactory in its results. To meet a possible criticism at this point, it might be mentioned that this procedure requires decidedly less skill than is needed by any professional engraver. Nothing more than care and practice is required to eliminate all significant error.

In only two respects is the technique that was used worthy of note. The pituitary can best be obtained by a special procedure. The lower jaw is first dissected off, then with a small long-bladed bone curette the tissues are scraped from the roof of the mouth until the sphenoid is laid bare. By means of the same curette the tympanic bullæ are loosened from their attachments and removed. The sphenoid is then disarticulated from the presphenoid by slight traction and carefully raised from the underlying structures. In case the dura mater remains adherent it is separated from the bone by means of an aurist's spud. Then with small curved scissors the area of the brain to which the pituitary is attached is circumsected and the cortical substance removed by means of an aurist's spoon to a watch glass of Ringer's solution, where the loosely adherent meninges are carefully teased off. With care this can be done without injury to the gland.

The pancreas in each case was removed to a dish of Ringer's solution, and the mesentery trimmed off. Then, after weighing, it was coiled into a disc upon a piece of filter paper, so that after fixation a median section could be cut to include the whole length of the gland. It was incised at short distances and dropped into fixitive, after which it retained its disc form.

Each gland was carefully weighed on a quick-acting balance, — the smaller to a tenth of a milligram, the larger to centigrams or milligrams, as indicated in the tables. In case the gland had to be kept any length of time — for example, while its mate was being dissected out — it was placed in Ringer's solution. All glands were weighed at as nearly as possible the same degree of dryness, — the superfluous moisture being removed by filter paper.

TABLE I.

GLAND WEIGHTS,[1] GUINEA PIGS,

Family No.	82		83			78			
Protocol No. . .	104	105	118	119	120	178	179	180	181
Sex	M	F	F	F	M	F	M	M	F
Weight, gm. . . .	120	98	87	102	93	75	75	69	73
Adrenals	.028	.034	.038	.033	.037	.031	.032	.029	.033
Hypophysis . . .	..	..	.0080	..	.0070	.0060	.0061	.0065	..
Heart	.50	.49	.52	.50	.54	.46	.48	.42	.46
Kidneys	.73	.84	.86	1.05	.96	1.17	1.08	1.13	.98
Liver	..	..	..	..	..	3.54	3.72	4.22	4.20
Ovaries	..	.0100	.0109	.0100	..	.0086	..	..	.0096
Pancreas	.13	.15	.22	.21	.26	.25	.29	.29	.25
Spleen	.08	..	.20	.16	.14	.12	.11	.12	.13
Testes	.053	..	..	..	.096	..	.076	.064	..
Thymus	.29	.32	.29	.28	.13	.30	.23	.28	.31
Thyroid	.025	.023	.029	.030	.031	.023	.027	.029	.021

[1] Expressed as percentages

The weights of the whole animals and of the various glands were found to vary in approximately the same proportions. By reducing the absolute weights, therefore, to percentages of the body weights a comparable series can be obtained. Table I throws some light on the validity of the method. It happens that the animals included in the table are arranged roughly according to the body weights, in a descending series, but no corresponding progression can be noted in the gland weights. In all cases therefore the weights have been expressed as percentages of the body weights.

To what extent the series would have to be carried to obtain satisfactory averages was not known. The effect of fortuitous variation had to be sufficiently eliminated to avoid confusion with any experi-

TABLE I.

NEWBORN, NORMAL.

214	248		276		294			295		238	Av.
227	249	250	285	286	291	292	293	299	300	419	...
M	M	M	F	M	M	F	M	F	M	M	...
114	60	89	52	47	60	61	61	70	65	60	77
.029	.015	.019	.021	.024	.024	.034	.025	.040	.038	.030	.030
.0044	.0060	..	.0058	.0074	.0065	.0057	.0052	.0049	.0046	.0063	.0060
.45	.52	.46	.50	.51	.60	.49	.52	.49	.54	.54	.50
.98	.77	.90	.92	.95	1.03	1.05	1.08	.93	.87	1.05	.97
4.68	5.20	4.64	4.75	5.95	4.40	4.30	4.09	4.70	5.47	5.01	4.59
..	..	..	.0077	..	..	.0072	..	.0117	..	..	.0095
.28	.13	.16	.19	.25	.22	.13	.21	.19	.22	.21	.21
.13	.11	.11	.11	.13	.12	.12	.13	.15	.15	.11	.13
.095	.067	.070	..	.106	.075	..	.085	..	.075	.091	.079
.20	.42	.27	.33	.28	.23	.28	.31	.16	.11	.21	.26
.026	.021	.022	.026	.024	.032	.026	.028	.031	.031	.032	.027

of body weights.

mental variation that might be secured. The matter was studied by plotting what may be called Curves of Averages by Continuous Summation, — using as abscissæ the number of individuals averaged, and as ordinates the average percentage weight calculated at each addition to the series. Obviously, when the curve becomes continuously horizontal, that is, when further additions have no appreciable effect, the fortuitous variation has become negligible, and as nearly a true norm has been established as is possible by the use of "averages." In all the curves an approximately uniform scale has been employed: 1 mm. space represents about 2 per cent of the normal value.

It was found that by the twentieth addition to the series, a fairly satisfactory horizontal had been reached. The average of this number was taken therefore as the norm. Table I gives the data from which the determinations were made, and Fig. 1, the curves plotted from this data by the method of continuous summation.

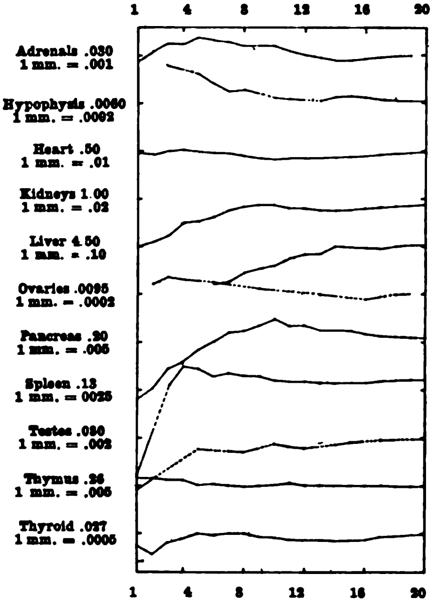

FIGURE 1. — Average weights of glands of normal new-born guinea pigs, platted by method of "averages by continuous summation." Abscissæ = average gland weights at each addition to the series. Ordinates = numbers of individuals arranged. Weights are expressed as percentages of body weights. One mm. on curves = 2 to 3 per cent of norm.

After having been weighed the glands were usually fixed in Bouin's formol-picro-acetic acid mixture; this was found to be the best of a number of fixitives tried, for routine work of this sort.

The tissues for histological study were sectioned in paraffin at 10 microns and stained for the most part with Mallory's aniline blue connective tissue stain. After Bouin fixation it is necessary, incidentally, to mordant the tissues on the slides with potassium dichromate. This procedure gives an excellent differentiation of the parts of such composite organs as the pituitary and the adrenals, and, in general, a fairly good histological picture. For finer details, however, slides were stained with Heidenhain's iron-hematoxylin and counterstained with orange G.

Congenital experimentation has not, so far as the writer is aware, been heretofore used in investigations of the nature proposed, and one of the purposes of the research was to determine its availability as a technical method. It is as yet largely unknown to what extent the body fluids of a pregnant mammal and of her fetus are similar in composition.[3] There is certainly, however, a tendency to chemical equilibrium between them, and it is probable that an experimental hyperthyroidism in the mother would cause a parallel condition in the offspring. It was hoped that the experimental conditions applied at this time of maximum plasticity would give the greatest possible morphological effect and that it would prove an additional advantage to throw the brunt of the experimental procedure onto a second animal better fitted than the newly born one itself to bear it.

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[3] For an extensive discussion of this question, *cf.* ZUNTZ: Der Stoffaustauc zwischen Mutter und Frucht, Ergebnisse der Physiologie; 1908, vii, p. 403.

There is little in the literature to indicate to what extent conditions affecting the internal secretions of the mother actually do produce a demonstrable effect in her offspring. Halsted,[4] in 1896, recorded that partial thyroidectomy of female dogs leads to marked congenital hypertrophy of the thyroids of their puppies. These results have been confirmed, doubtfully, by Edmunds [5] on a dog, and by Hunt [6] in case of guinea pigs. Lanz,[7] however, was unable to get such an effect in a dog and two goats. Ceni [8] has noted that chicks hatched from eggs laid by thyroidectomized hens usually show anomalies of development. He interprets this as a case of "hereditary effect, of secondary origin," but it is rather more likely due to an abnormal chemical composition of the eggs, due in turn to hypothyroidism in the hens. The clinical literature has not afforded more definite information. The children of goitrous mothers often show trophic disturbances, such as rachitis, suggesting disturbed conditions of internal secretion,[9] but just what these conditions specifically are does not appear.

From the fact that the symptoms of hypothyroidism are clearly recognized, and can be obviated by the ingestion of thyroid substance, there is little or no question that thyroid feeding is a valid method of obtaining a true condition of hyperthyroidism.

In the "congenital hyperthyroidism" series, twenty-eight pregnant guinea pigs were treated with thyroid substance in different doses, and for various lengths of time, as indicated in Table II following. The commercial desiccated sheep's thyroid of Parke Davis & Co. was used. The material, suspended in water, was fed to the animal with a pipette. In most cases this was done daily; in some instances,

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[4] HALSTED: Johns Hopkins Hospital reports, 1896, i, p. 373.

[5] EDMUNDS: Lancet, 1901, Pt. 1, p. 1451.

[6] HUNT: Journal of the American Medical Association, 1907, xlix, p. 1323.

[7] LANZ: Beiträge zur klinische Chirurgie, 1909, xlv, p. 208.

[8] CENI: Archives italiennes de biologie, 1905, xlii, p. 420.

[9] SCHMAUCH: American journal of obstetrics and diseases of women and children, 1909, lx, p. 1.

TABLE II.

MOTHERS ON THYROID DIET.

Protocol.	Dose.	Time.	Total Dose.	Results.
No.	Gm.	Days.	Gm.	
56	0.2–0.025	51	2.55	Delivered 3, Nos. 175–177.
75	0.10	13	0.13	Died, thyroidism.
84	.. .	17	..	Died, thyroidism.
87	0.10	8	0.80	Died, thyroidism.
88	0.05	4	0.20	Delivered 2, Nos. 135–136.
91	0.25	8	2.00	Died, thyroidism.
146	0.025	7	.175	Died, thyroidism.
149	0.10	15	1.50	Aborted.
151	0.025–0.10	13	0.70	Delivered 3, Nos. 182 and 2 stillborn.
154	0.025–0.05	22	.85	Delivered 3, Nos. 207–209. All stillborn.
155	0.05–0.1	16	1.60	Died, thyroidism.
164	0.1	11	1.10	Died, thyroidism.
165	0.025–0.015	42	0.55	Aborted.
169	0.015	22	0.33	Delivered 2, Nos. 369–370.
171	0.05–0.025	36	0.94	Delivered 2, Nos. 406–407.
191	0.02	9	0.18	Delivered 3, Nos. 288–290.
217	0.05	4	0.20	Aborted.
232	0.05	8	0.40	Delivered 3, Nos. 278–279, 1 stillborn.
233	0.015	22	0.33	Aborted.
235	0.025	9	0.225	Aborted.
236	0.025	9	0.225	Died, thyroidism.
237	..	..	..	Aborted.
238	0.020	10	0.20	Aborted.
252	0.025	20	0.50	Died, thyroidism.
256	0.025	6	0.150	Aborted.
261	0.015	9	0.135	Delivered 1, No. 287.
296	0.015 with rests.	40	0.49	Delivered 2, Nos. 408–409.
297	0.010	8	0.080	Died, thyroidism.

however, longer intervals were employed. In most instances the treatment was continuous, but the effect of alternating periods of treatment with those of rest was also tried. The essential details of the experiments are set forth in the table. An inspection of the table shows that idiosyncrasy plays a large part in such experiments. Animal No. 297 succumbed to a dose of 0.01 gm. per day for eight days, while animal No. 91 survived for the same length of time a dose twenty-five times as great. In the animals that survived, abortions were frequent. In most cases 0.015 gm. per day could be borne, but even this dose was too great in case of animal No. 233. The greatest dosage that produced neither death nor abortion was in case of No. 56, in which the amount varied from 0.2 gm. for a few doses at the beginning to 0.025 gm. per day at the end. The method of continuous treatment was found more efficacious than discontinuous, but more likely also to cause death or abortion.

From the mothers in this series there were obtained twenty-one offspring. With the exception of one family, all the animals were born alive and were apparently perfectly normal in every way except as to the size of the glands considered; family No. 154 was still-born, but showed, otherwise, every evidence of being quite as nearly normal as the others. The weights of their organs are tabulated in the order of the total amount of thyroid given to each mother (Table III). Since no account is taken of the weights of the mothers, however, the results are only approximately quantitative. Within the families the columns have been arranged in a descending series, according to the weights of the thyroid glands.

In this particular series the averages of the gland weights are not very significant *as such*, on account of the preponderance of cases in which the dosage had been too slight to exert a demonstrable effect.

Histological studies were made of the material from Protocols Nos. 136, 278, 406, 408, and 409 from the experimental series, and from Nos. 118, 120, and 285 as "normals."

To summarize the results of the experiments, it appears that as an effect of thyroidism in the mother, the weights of the adrenals were depressed from the normal. In the offspring of mother No. 56 which had received the greatest amount of thyroid substance, the average depression of these glands was 53 per cent. Histologically they appeared normal in every way, except that the lecithin (?) globules of

TABLE III.

GLAND WEIGHTS,[1] GUINEA PIGS,

Family	261	191			88		169		232	
Dose, gm.	.135	.18			.20		.33		.40	
Protocol. No.	287	288	289	290	135	136	370	369	279	278
Sex	F	M	M	M	F	M	F	M	M	F
Weight, gm.	100	68	80	73	31	63	70	70	77	70
Adrenals	.029	.029	.029	.031	.025	.017	.036	.041	.021	.021
Hypophysis	.0041	.0050	.0048	.0055	..	.0071	.0057	.0067	.0043	.0041
Heart	.46	.42	.43	.42	.45	.43	.46	.47	.52	.50
Kidneys	.91	.78	.85	1.01	.97	.82	.84	.71	.82	.87
Liver	5.19	5.16	4.82	4.13	4.90	4.31	4.86	4.11	5.14	5.97
Ovaries	.0065	..	..	..	.0110	..	.0061	..	..	.0087
Pancreas	.22	.19	.19	.22	..	.16	.16	.18	.24	.20
Spleen	.09	.11	.12	.12	.12	.13	.08	.07	.09	.08
Testes	..	.090	.094	.100	..	.071	..	.070	.094	..
Thymus	.35	.32	.34	.43	.68	.64	.30	.31	.22	.32
Thyroid	.028	.029	.028	.027	.032	.028	.030	.024	.032	.026

[1] Expressed as percentages

the cortical cells were rather less prominent in the experimental series. By inspection no differences could be recognized in pigmentation, in the number of mitoses, or in the proportions between cortex and medulla.

A possible explanation of these results is that the depression was a direct reaction to excess of thyroid substance in the maternal-fetal body fluids. Such an interpretation, however, would be opposed to the conclusions of Bruckner[10] and of Rudinger, Falta, and Eppinger[11]

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[10] BRUCKNER: Comptes rendus société de biologie, 1908, lxiv, p. 1123.

[11] RUDINGER, FALTA, and EPPINGER: Zeitschrift für klinische Medicin, 1908, lxiv, p. 1.

TABLE III.

NEW-BORN, CONGENITAL THYROIDISM.

296		151	154			171		56			Av.	Norm. Av.
.59		.70	.85			.94		2.50				
408	409	182	208	209	207	406	407	176	175	77	..	..
M	M	F	M	M	F	M	F	F	F	M	..	..
65	67	86	57	61	51	60	56	58	55	57	65	77
.024	.030	.023	.020	.018	.018	.020	.032	.014	.014	.012	.024	.030
.0060	.0058	.0053	.0080	.0070	.0059	.0053	.0054	.0067	.0045	.0063	.0054	.0060
.50	.45	.48	..	.50	.56	.46	.50	.38	.35	.42	.46	.50
.80	.84	.78	1.00	1.08	.94	.89	1.05	.87	.83	.83	.89	.97
5.63	4.75	6.21	..	..	..	3.90	4.13	4.15	4.02	4.81	5.13	4.59
..	..	.0059	..	..	..	..	.0077	.0055	.0049	..	.0070	.0095
.26	.21	.15	.20	.22	.25	.24	.25	.18	.20	.17	.21	.21
.10	.14	.11	.18	.17	.12	.09	.11	.11	.10	.11	.11	.13
.058	.067	..	080	.070	.074	.110	..	..	..	.056	.079	.079
.50	.52	.38	.20	.47	.33	.30	.30	.53	.62	.63	.41	.26
.020	.023	.018	.018	.017	.014	.020	.018	.013	.012	.012	.022	.027

of body weights.

that the thyroid stimulates the adrenals. It is possible to account for the adrenal depression in harmony with their views on the supposition that the adrenals of the mother had been stimulated by the thyroid substance, and a condition of adrenalism thus produced in both maternal and fetal organisms. The adrenals of the offspring reacted to this condition by hypoplasia, and a decreased production of the fat-like secretion of the cortex. This latter result harmonizes with Marenesco and Parhon's [12] conclusion that the formation of this substance is especially influenced by thyroid conditions.

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[12] MARENISCO et PARHON: Comptes rendus société de biologie, 1908, lxiv, p. 768.

The results as regards the hypophysis were inconclusive. In family No. 56 there was a depression of about 3 per cent only, but the average for the whole series was 10 per cent below the normal. The variability of the weights of the pituitaries of the series was so great that a deviation of this magnitude is not very significant. No differences at all between the normal and the experimental glands could be recognized histologically. Eosinophile cells were notably numerous in both; about 50–60 per cent of the anterior lobe was made up of this type. No colloid was observed in any of the glands. In view of the close correlation that supposedly exists between the thyroid and the pituitary, the negative results obtained were hardly expected.

The ovaries showed a considerable depression from the normal. In the extreme case — Family No. 56 — the average depression was 45 per cent. Sufficient material for histological study was not available to permit significant conclusions. In the normal cases studied, however, oocyte formation was further advanced, and mitoses in the follicular cells more numerous than in the experimental case. An explanation similar to that suggested in regard to the adrenals would account for the ovarian depression; *i. e.*, that it was a hypoplasia due to excess of ovarian secretion produced in the mother by the thyroid medication.

The pancreas was not demonstrably affected. Particular attention was paid to the islands of Langerhans, but no difference could be observed in their size, numbers, constituent cells or frequency of mitoses.

No demonstrable difference occurred either in the weights or histological character of the testes.

The thymus showed an average hypertrophy of 38 per cent. In Family No. 56, the average weight was 127 per cent above normal. Histological study indicates that the hypertrophy was largely or entirely confined to the cortical tissue which was proportionately in excess. The mitoses which were notably more numerous in the experimental material were mostly confined to the cortical zone. No difference was observed in the corpuscles of Hassal. Unless it be assumed that the maternal thymus is normally functional and that this function was depressed by the excess of thyroid, there is no apparent explanation for the hyperplasia of the organ except as due to a direct stimulating effect of the thyroid material. Since there is no nervous connection between the mother and fetus, it must be a pure hormone reaction.

This condition is in harmony with the frequent clinical finding of hypertrophied or persistent thymus in Graves' disease. The reaction may be worth investigating further, for its bearing on the etiology of *status lymphaticus*, in which thymus hypertrophy and adrenal hypoplasia are prominent features.

The thyroid weights in the experimental series were also depressed; in Family No. 56 the average weight is 56 per cent below normal. Histologically, the normal and experimental glands were rather similar in appearance. Colloid was equally abundant in both. The cells lining the alveoli, however, were somewhat shorter than normal. Since, as has been noted by Halsted[4] and others, increased activity of the thyroid is marked by a notable increase in the height of the alveolar cells, the histological appearance as well as the decreased weight indicate depressed functioning in the glands of the experimental series. The hypoplasia of the thyroids was in all probability simply a compensatory reaction to the experimental hyperthyroidism, just as the hypertrophy noted by Halsted after thyroidectomy was a direct reaction to hypothyroidism.

It is interesting to note that in the "normal" family, No. 248, in which spontaneous thyroid depression occurred, a hypoplasia of the adrenals and a slight hypertrophy of the thymus also were found.

The explanation offered in case of the adrenals suggests the chief objection to congenital experimentation as a method. It is apparently in some degree fitted to demonstrate the existence of relationships between organs, but, except in the case of the testes, which do not occur in the mother, an unequivocal explanation of any results secured is not possible. They might be a direct effect of influences acting upon the fetal structures, or a secondary effect of influences upon the homologous maternal structures. The two possibilities lead to exactly opposite conclusions.

Two other objections suggest themselves. The abnormal gland weights that were found in the experimental animals might be due either to premature birth or to a selective wasting effect of thyroid medication upon the body tissues. There is reason to think, however, that neither is valid. Jackson[13] has recently published the results of an extensive investigation of the percentage weights or fetal glands at various ages, and his curves show that no such deviations occur shortly before birth as were found in the present series. His results are based upon human material, but they are probably of general applicability. The fact that the thymus hypertrophied militates against the second objection,.in that this gland is remarkably subject to atrophy, in conditions of general emaciation.[14]

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[13] JACKSON: American journal of anatomy, 1909, ix, p. 119.

The method has the distinct advantage that, as regards the end effects, direct nervous influences are excluded. Any results secured, therefore, must be explained ultimately upon a hormone basis.

My thanks are due to Prof. W. B. Cannon for his constant interest in the progress of this work and for many helpful criticisms.

[14] HOWELL: Text-book of physiology, 3d edit., Philadelphia, 1909, p. 840.

THE PROPHYLACTIC ACTION OF ATROPIN IN IMMEDIATE ANAPHYLAXIS OF GUINEA PIGS. — THIRD COMMUNICATION.

By J. AUER.

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INTRODUCTION.

ABOUT one year ago it was stated in a preliminary note[1] that atropin sulphate could prevent the death of a guinea pig from a very acute type of anaphylaxis which we later termed the immediate anaphylactic reaction.[2] We first used atropin because the experimental facts which we obtained in our investigation all pointed to the conclusion that the bronchial muscles, especially those of the finer tubes, played an important, if not the most important, rôle in the production of the pulmonary stenosis which led swiftly up to the death of the animal by asphyxia.[3] On the basis of this conclusion, which has since been adopted by Anderson and Schultz[4] and by Biedl and Kraus,[5] it was natural that atropin should be employed because of its well-known paralyzing action upon the vagus endings in the bronchial muscles. This action of atropin was first shown by Dreser[6] and was later corroborated by Beer,[7] Einthoven,[8] and by Dixon and Brodie.[9] The therapeutic results we obtained with this alkaloid in an experimental test were gratifying and were reported in our preliminary note. Since that time some series of experiments have been carried out in guinea pigs of varying sensitiveness, and these results will be briefly reported in the following pages.

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[1] Auer and Lewis: Journal of the American Medical Association, 1909, viii, p. 458.

[2] Auer and Lewis: Journal of experimental medicine, 1910, xii, p. 153.

[3] Auer and Lewis: *Ibid.*, pp. 165–169.

[4] Anderson and Schultz: Proceedings of the Society for Experimental Biology and Medicine, 1909, vii, p. 34.

[5] Biedl and Kraus: Wiener klinische Wochenschrift, 1910, xxiii, No. 11, p. 386.

[6] Dreser: Archiv für experimentelle Pathologie und Pharmakologie, 1890, xxvi, p. 255.

[7] Beer, Archiv für Physiologie, 1892, Supplement-Band, p. 150.

[8] Einthoven: Archiv für die gesammte Physiologie, 1892, li, p. 428.

[9] Dixon and Brodie: Journal of physiology, 1903, xxix, pp. 162, 168.

EXPERIMENTS.

In order that a clear conception of the course of these experiments may be obtained, two specimen protocols will be given which may serve as types, and the experiments, which will be presented later in tabular form, were carried out in this fashion.

Control Experiment, June 30, 1910. — G. pig, male, F 16; 700 gm. Sensitized Nov. 17, 1909, by 1 c.c. H. S. subc.

10.27. Stretched on electric pad at L. Start ether.

10.29. Cannula in external jugular vein; stop ether.

10.31. Rectal temperature 38.8°.

10.35. 0.5 c.c. 10 per cent heated H. S. jug. vein; 1½ c.c. Ringer sol.

10.36. Chest sinks in fairly well with inspiration. Respiration slowed.

10.36½. Chest sinks in strongly with each inspiration. Respiration slow; struggles, no sound audible.

10.37. Chest sinks in strongly with inspiration; tongue bluish; struggles, choked squeak.

10.39. Short convulsions; no respiratory sound audible.

10.40. Mouth opens with inspiration; chest movements getting less and less; visible peristalsis.

10.41. No respiration. Rectal temperature 38.7°.

Autopsy: Lungs typical, large, full, pale bluish pink; left upper lobe only slightly distended; no difference between two sides otherwise.

Atropin. June 30, 1910. — G. pig, male, F 19; 700 gm. Sensitized Nov. 17, 1909, by 1 c.c. H. S. subc.

11.40. Stretched on electric pad at L. Start ether.

11.44. Cannula in external jugular vein; stop ether.

11.50. Rectal temperature 39.9°.

11.51. 2 mg. atropin sulph. jug. vein (1 per cent solution). 0.5 c.c. 10 per cent heated H. S. jug. vein; 1½ c.c. Ringer sol.

11.52½. Slight sinking in of chest with inspiration.

11.53. Sharp struggle with some rather choked squeaks.

11.55. Slight sinking in only of chest; sharp struggles with choked squeaks.

11.56. Same; during struggles air heard issuing from mouth and nose.

11.57. Same; only slight sinking in of chest; respiration moderately rapid.

11.59. Chest sinks in a bit more. 1 mg. atropin, jugular vein; 1 c.c. Ringer sol. to wash out cannula.

12.05. Chest sinks in only very moderately with inspiration. Wound washed with 2.5 per cent carbolic sol. Sutured; squeaks slightly as needle passes through skin. Rectal temperature 40.4°. Placed in box.

12.08. Moves about box; holds head up; hair of head and neck erect, smooth over rest of body. Respiration rapid. Occasionally hind legs straighten abruptly and raise rump of animal.

1.20. Good condition.

3.40. Good condition.

July 1, 10 A. M. Lively and active.

July 7. Well, lively and active.

The protocols quoted above show well that the lungs are markedly involved in immediate anaphylaxis and that atropin reduces these manifestations strongly. Yet the significance of the protocols is not, perhaps, obvious, and for this reason a graphic record of the lung changes themselves is given. Fig. 1 shows these changes very well. This tracing was obtained from a guinea pig which had been sensitized June 4, 1909, by the subcutaneous injection of 5 c.c. of meningitis serum. On September 27, 1909, this animal was stretched out on an electric pad, etherized, both vagi cut; a pleural cannula fixed in the right pleural cavity; 2.5 mg. curarin injected into the external jugular vein and artificial respiration started. The volume changes of the lung were recorded by connecting the pleural cannula with a Marey tambour, upstroke of the writing lever being caused by inflation of the lung. Both vagi were first stimulated to cause a bronchial tonus (not reproduced in tracing). Then 0.6 c.c. of anti-meningitis (horse) serum was injected into the jugular vein and the cannula washed clear by 1 c.c. of saline solution. Within a few seconds (time is marked in four-second intervals on tracing) and before the salt solution is injected, a well-marked increase in the lung volume oscillations occurs, a broncho-dilatation,[10] which is followed swiftly by a broncho-constriction of such a degree that the air blasts from the artificial respiration machine hardly cause any volume changes in the lung, although the volume, force, and rate of the air delivery has in no wise been altered. The animal now receives practically no air, and if it were not curarized would show powerful struggles followed swiftly by asphyctic convulsions and death.[11] In the tracing under consideration, however, conditions were not allowed to progress to this pass, but 3 mg. of atropin were injected into the jugular vein. Very swiftly, a matter of seconds, the volume changes of the lung, due to the artificial respiration, begin to increase, showing that the pulmonary stenosis, which had been produced by the horse serum, was slowly yielding to the atropin. A second injection of atropin, this time 2 mg., was given, perhaps unnecessarily, to hasten the removal of the stenosis, and two and one-half minutes after the first atropin injection the lung again expands and collapses to artificial respiration even better than before the injection of the toxic dose. This tracing (Fig. 1) shows at a glance the kernel of this communication.

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[10] Auer and Lewis: Journal of experimental medicine, 1910, xii, pp. 167, 168. This has also been observed by Biedl and Kraus: *Loc. cit.*, p. 386.

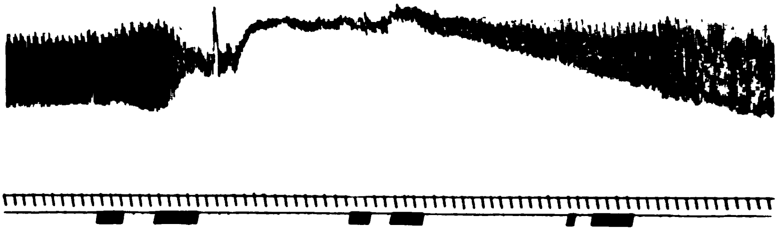

FIGURE 1. — From the right pleural cavity of a sensitized curarized guinea pig September 27, 1909. Upstroke = inflation of lung under artificial respiration from a machine. Time = four-second intervals. At once after the intravenous injection of 0.6 c.c. of horse serum (first black band), a short but well-marked broncho-dilatation occurs. This is swiftly followed by an almost maximal broncho-constriction. During this broncho-constriction 3 mg. atropin and again 2 mg. were injected into the jugular vein. Note prompt return of lung oscillations.

While this tracing shows well the vital functional interference which horse serum causes in a sensitized guinea pig and its removal by atropin, there is nothing which conveys to the eye the marked gross anatomical change which accompanies this alteration of lung function.[12] In order to show this and also the effect of atropin, two guinea pigs from the

[11] See Plate VIII, AUER and LEWIS: *Loc. cit.*, for a graphic registration of this.

[12] AUER and LEWIS: *Loc. cit.*, p. 157.

same lot, sensitized by the subcutaneous injection of 1 c.c. of horse serum on November 17, 1909, received on January 24, 1910, intravenously 0.3 c.c. of a 10 per cent solution of heated horse serum.[13] One of these guinea pigs had received 3 mg. of atropin subcutaneously about fourteen minutes before the toxic dose of horse serum. The control animal died with typical respiratory symptoms in five minutes, while the atropin animal showed practically no respiratory involvement during ten minutes of observation, when it was killed by section of the medulla. The lungs of these animals were excised and photographed. Fig. 2 is a reproduction of this photograph. Inspection of this picture shows that the difference between the two lungs is striking: The control lungs (*b* in the picture) are pale, full, and light of weight; they seem to be fixed in an inspiratory condition in spite of the fact that they are excised from the thorax and that there is no mechanical obstruction in the trachea; moderate tracheal inflation would exert no effect on the lung volume.[14] They are full of air which cannot escape, and inspection of the lung surface with the naked eye shows beautifully the distended alveolar air-sacs. If the lung surface had been pricked by a needle (which was done in numerous other experiments), air mixed with a little blood would have been seen bubbling out as if under some tension. In short, the lungs of this control animal look exactly like normal lungs at the end of a full inspiration;[15] the only difference is that these anaphylactic lungs maintain this inspiratory condition when removed from the thorax, the atmospheric pressure and the pull of the stretched elastic tissue, which strain to empty the lung, being counteracted by a stenosis of the pulmonary air passages.[16] These lungs again indicate clearly why the animal died of asphyxia. On the other hand, the lungs of the atropin animal (*a* in Fig. 2) show a very different appearance: they are collapsed, small, and almost air-less, about one half the size of the anaphylactic lungs; their color is a dark gray, and the surface of the lungs shows numerous fine wrinkles (not well brought out in the photograph); on immediate moderate rhythmic inflation these lungs would expand and collapse readily; in short, these atropin lungs look like those which may be seen in any normal guinea pig shortly after death when the thorax is opened. There is one exception, however, to be noted; the right middle lobe looks pale and distended, and this lobe resembles the lungs of the control animal; this lobe is fixed in a more or less inspiratory condition and contains a good amount of air; the significance of this partial inspiratory immobilization will be considered in another place. From this description it may readily be seen that the impairment of lung function which horse serum produced in the sensitized guinea pig had been successfully decreased by atropin so that it no longer menaced the life of the animal; here atropin exerted a curative effect.

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[13] It must be added that in both animals the right vagus had been resected on January 11. I have shown in another place (Proceedings of the Society of Experimental Biology and Medicine, 1910, vii, p. 104) that this has no effect on immediate anaphylaxis.

[14] AUER and LEWIS: *Loc. cit.*, p. 162; also BIEDL and KRAUS: *Loc. cit.*, p. 386.

[15] For a fuller description of the typical anaphylactic lung see AUER and LEWIS: *Loc. cit.*, p. 156.

[16] The production of this stenosis is shown in Fig. 1.

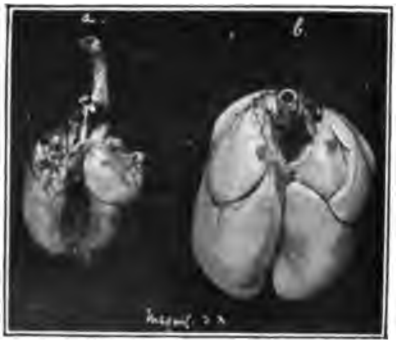

Figure 2. — Lungs from two guinea pigs of the same lot. Magnification about 2 X. Both guinea pigs were sensitized November 19, 1909, by the subcutaneous injection of 1 c.c. horse serum. On January 11, 1910, the right vagus was resected in the neck of each. On January 24, 1910, the animal, whose lungs are marked *a* in the photograph, received 3 mg. of atropin subcutaneously; fourteen minutes later the toxic dose, 0.3 c.c. of a 10 per cent solution of heated horse serum, was injected into the jugular vein; ten minutes later the animal was killed by section of the medulla. The lungs were then excised and photographed together with those of the control animal. The control (marked *b* in photograph) received the same dosage of horse serum, but no atropin. It died with typical respiratory symptoms within five minutes after the injection.

In the foregoing pages proof of various kinds has been submitted that atropin may prevent or abolish the death-producing inspiratory immobilization of the guinea pig's lungs in immediate anaphylaxis. There remains to be shown that this life-saving action of atropin occurs in a good percentage of the cases where this drug is used, and for this purpose a few tables will be presented

ATROPIN STATISTICS.

All the guinea pigs whose records will form one table belong to the same lot and are practically of the same age and weight. This holds true also of Table I, where the guinea pigs were sensitized at two different times and with different amounts of serum. The course of each experiment is shown by the two protocols quoted on preceding pages. Atropin was always used in the form of a sulphate and in a 1 per cent solution; when injected subcutaneously, it was given in the right upper abdominal quadrant and eleven to fifteen minutes allowed to elapse before the injection of the toxic dose of serum. All intravenous injections were made into a cannula ligated in the external jugular vein, the injection of atropin or serum being followed by 1 c.c. of saline or Ringer solution to wash out the cannula. The horse serum employed was almost always heated to 55° for thirty-five minutes to reduce its toxicity, and the dose was about the minimal lethal dose. The slight operation required for this work was done under primary ether anæsthesia as a rule, and about five minutes were allowed to

TABLE I.

ANIMALS SENSITIZED FOURTEEN TO SIXTEEN DAYS. AVERAGE WEIGHT, 400 GM.

No.	Sensitized.	Date of toxic dose.	Atropin.	Toxic dose.	Result.
E 5	Nov. 17, '09. 1 cc. H.S. subc.	Dec. 3, '09. 16 days	3 mg. subc.	0.6 cc. heated H.S. jug. vein.	Slight symptoms. Recovery.
E 4	" "	" "	None.	" " "	Death in 5 min.
E 6	" "	" "	3 mg. subc.	" " "	Slight symptoms. Recovery.
E 102	March 14 1 c.c. 10% H.S. subc.	March 28 14 days	None.	0.4 c.c. heated. H.S. jug. vein	Death in 4 min.
E 103	" "	" "	3 mg. subc. 1½ mg. jug. vein.	" " "	Death in 6 min.
E 104	" "	" "	None.	0.3 c.c. heated H.S. jug. vein.	Death in 9 min.
E 105	" "	" "	5 mg. subc. 1½ mg. jug. vein.	" " "	Death in 75 min.
E 107	" "	" "	None.	" " "	Slight symptoms. Recovery.
E 108	" "	" "	5 mg. subc. 1 mg. jug.	0.4 c.c. heated H.S. jug. vein	Slight symptoms. Recovery.
E 110	" "	" "	None.	" " "	Death in 4 min.
E 109	" "	" "	4 mg. Subc. 3 mg. jug.	" " "	Good chest symptoms. Recovery.

6 atropin pigs: 2 died = Death 33% = Recovery 66%.
5 controls : 4 died = Death 80% = Recovery 20%.

TABLE II.

ANIMALS SENSITIZED TWENTY-ONE TO TWENTY-SEVEN DAYS. AVERAGE WEIGHT, 300 GM.

No.	Sensitized.	Date of tox. dose.	Atropin.	Toxic dose.	Result.
D 131	Oct. 1, 1909. 1.c.c. H.S. subc.	Oct. 22, 21 days		0.5 c.c. heated H.S jug. vein	Death in 5 min.
D 132	" "	" "	1 mg. subc.	" "	No chest symptoms. Recovery.
D 133	" "	" "		" "	Mod. chest symptoms. Recovery.
D 134	" "	" "	1 mg. subc.	" "	Death in 4 min.
D 135	" "	24 days		" "	Death in 4 min.
D 136	" "	" "	3 mg. subc.	" "	Slight symptoms. Recovery.
D 138	" "	" "		" "	Moderate symptoms. Died during night.
D 137	" "	" "	3 mg. subc.	" "	Slight respiratory symptoms. Recovery
D 139	" "	" "		" "	Death in 4 min.
D 140	" "	" "	3 mg. subc.	" "	No symptoms. Recovery. Lively next day.
D 141	" "	25 days		" "	Good respiratory symptoms. Recovery.
D 143	" "	" "	3 mg. subc.	" "	No symptoms (resp.) during 11 min. Killed to study lungs.
D 142	" "	" "		" "	Died in 4 min.
D 144	" "	" "	3 mg. subc.	" "	Slight respiratory symptoms after 14 min. Killed to study lung.
D 145	" "	" "		" "	Death in 5 min.
D 146	" "	26 days		" "	Death in 4 min.
D 148	" "	" "	3 mg. subc.	" "	Death in 22 min. Very sick, but only mod. resp. symptoms.
D 147	" "	" "		" "	Death in 6 min.
D 149	" "	" "	3 mg. subc.	" "	Very slight respiratory symptoms. Recovery.
D 150	" "	27 days		0.4 c.c. heated jug. vein	Death in 5 min.
D 151	" "	" "	3 mg. subc.	" "	Very slight resp'y symptoms after 9 min. Killed to study lungs.
D 154	" "	" "		0.5 c.c. heated H.S. j. v.	Death in 5 min.
D 155	" "	" "	3 mg. subc.	" "	No respiratory symptoms during 15 min. Killed to study lungs.

11 atropin pigs : 2 died = Death 18% = Recovery 82%.
12 controls : 9 died = Death 75% = Recovery 25%.

pass after stoppage of the ether to permit full recovery from the effects of the anæsthetic. The loss of heat, which occurs swiftly in such a small animal as the guinea pig when it is stretched out at full length, was prevented by placing the animal on an electric heating pad. It will be noted that for practically every atropin experiment there is a control experiment.

TABLE III.

ANIMALS SENSITIZED TWO HUNDRED AND TWENTY-FOUR TO TWO HUNDRED AND TWENTY-SIX DAYS. AVERAGE WEIGHT, 700 GM.

No.	Sensitized.	Date of tox. dose.	Atropin.	Toxic dose.	Results.
F 11	Nov. 17, 1909. 1 c.c. H.S. subc.	June 29, '10. 224 days	....	0.3 c.c. 10% h'd H.S. j. v.	Death in 7 min.
F 13	" "	" "	3 mg. subc. 1 mg. j. v.	" "	Slight respiratory symptoms. Recovery.
F 14	" "	" "	....	" "	Moderate respiratory symptoms.
F 12	" "	" "	3 mg. subc. 2 mg. j. v. 1 mg. j. v.	" "	Death in 3 min.
F 16	" "	225 days	....	0.5 c.c. 10% h'd H.S. j. v.	Death in 6 min.
F 19	" "	" "	3 mg. j. v. 1 mg. j. v.	" "	Slight resp. symptoms. Recovery.
F 21	" "	" "	....	" "	Death in 13 min.
F 17	" "	" "	3 mg. subc. 4 mg. j. v. (4 doses.)	" "	Death in 14 min.
F 24	" "	" "	....	" "	Moderate resp. symptoms. Recovery.
F 22	" "	" "	2 mg. j. v. 1 mg. j. v.	" "	Death in 2 min.
F 23	" "	" "	2 mg. j. v.	" "	No resp. symptoms. Lively next day.
F 26	" "	226 days	....	" "	Death in 3 min.
F 27	" "	" "	2 mg. j. v.	" "	Very slight symptoms. Recovery. Lively next day.
F 30	" "	" "	....	" "	Death in 4 min.
F 28	" "	" "	2 mg. j. v. 1 mg. j. v.	" "	Mod. resp. symptoms. Recovery.

8 atropin pigs : 3 dead = Death 38% = Recoveries 62%.
7 controls : 5 dead = Death 71% = Recoveries 29%.

Whenever "recovery" is noted in the protocols it means that the animal was observed at least two days after the experiment.

Four atropin animals which showed practically no respiratory symptoms were killed after nine to fourteen minutes in order to study their lungs. As the controls usually died within five minutes showing marked respiratory involvement, these four animals are classed as recoveries.

DISCUSSION.

From the preceding tables it will be seen that atropin exerts a clear-cut prophylactic effect in a very acute type of serum anaphylaxis which Lewis and I have termed immediate anaphylaxis. Out of twenty-five guinea pigs which had been sensitized from fourteen to two hundred and twenty-six days, atropin saved eighteen, or 72 per cent, while out of twenty-four guinea pigs which served as controls for the atropin series, only six survived, or 25 per cent. Stated otherwise, the death rate in the atropin series was 38 per cent, while in the control series it was 75 per cent. These results substantiate well the statement made in our preliminary report.[17]

Since this preliminary report first appeared, the effect of atropin in serum anaphylaxis was tested by Anderson and Schultz [18] and by Biedl and Kraus.[19] Anderson and Schultz succeeded in saving only about 28 per cent of their animals (4 out of 14) by the use of atropin. These observers used young guinea pigs weighing about 300 gm. after they had been sensitized by an intra-orbital injection of 0.01 c.c. of horse serum for twenty-one to thirty days. The toxic dose was 0.5 c.c. of the same serum injected intravenously. Atropin was given "usually intraperitoneally" in 3 mg. doses. They do not state how much time was allowed to elapse between the injection of the atropin and the administration of the toxic dose of serum. This latter point is of importance because the effect of atropin on the lungs is by no means permanent. There is a certain optimum time for the injection of the toxic dose after atropin has been given, and we may with justice assume that this is apparently when the balance between the absorption and excretion of the drug is such that its concentration in the blood is greatest. This time naturally will vary with the mode of administration of the alkaloid. As I have no experience of my own with the prophylactic effect of atropin when given intraperitoneally, it would be useless for me to speculate on the causes for the meagre yield of therapeutic results in the hands of Anderson and Schultz.

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[17] AUER and LEWIS: Journal of the American Medical Association, 1909, viii, p. 458.

[18] ANDERSON and SCHULTZ: Proceedings of the Society for Experimental Biology and Medicine, 1909, vii, p. 35.

[19] BIEDL and KRAUS: Wiener klinische Wochenschrift, 1910, xxiii, p. 387.

The other observers who used atropin were Biedl and Kraus. They injected the drug intravenously in guinea pigs, the doses varying from 1 to 10 mg., and were successful in abolishing the anaphylactic immobility of the lung. They also found that 5 mg. given prophylactically prevented the appearance of lung symptoms, but these investigators apparently have not tested what percentage of success they could obtain in the attempt to save sensitized guinea pigs from the fatal effect of serum when injected the second time.

Mode of administration of atropin.— In earlier work atropin was always given subcutaneously some ten minutes before the toxic dose of serum. This mode of incorporating the drug has the drawback that there is no definite knowledge when the lung is most under the influence of the alkaloid, for it is impossible to tell in the ordinary experimental test when the ratio between absorption of the alkaloid and its excretion has reached that point where the blood contains most of the substance. This problem is still further complicated by the fact that the "subcutaneous" injections are often partially intradermal or intramuscular, combinations which vitally affect the rate of absorption. Now, since the rôle of atropin and toxic dose of serum is that of antagonists, at least as far as the bronchial muscles are concerned,[20] it is obvious that the most favorable time for injection of the toxic dose is when the blood contains most of the atropin. For this reason I have lately used the intravenous route for the injection of the prophylactic dose of atropin. As a rule 2 mg. of the drug in a 1 per cent solution is injected into the external jugular vein and the cannula washed out by 1 c.c. of Ringer solution. Now after the lapse of ten to fifteen seconds the toxic dose of horse serum is injected also intravenously. Done in this fashion, the experimenter is certain that the maximum amount of the atropin swiftly comes within striking distance of the bronchial muscles, and moreover he is fairly sure of the time when this occurs. Another advantage of this method is that further injections of atropin may swiftly be sent to the bronchial muscles, if the animal shows the characteristic chest signs that the toxic dose of serum is gaining the upper hand. Care must, however, be exercised not to send in too much atropin, for an excess of this drug will paralyze the respiratory centre. I feel certain that in a number of cases I gave too much atropin, and thus aided in killing the animal when I was attempting to save it. The drug also should not be injected too swiftly nor in too concentrated a form.

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[20] I have demonstrated recently that the bronchial muscles themselves are sensitized (Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, p. 104), and that atropin paralyzes the bronchial muscles and not only the nerve endings (Journal of experimental medicine, 1910, xii, No. 5).

Toxic dose. — In order to demonstrate the prophylactic effect of atropin in immediate anaphylaxis, I have always attempted to use the minimal lethal dose of horse serum for the second or toxic injection. This was done so that the effect of atropin could be observed most clearly with the smallest dose of the drug. I have made no attempt so far to establish how many lethal doses of horse serum a sensitized guinea pig will stand with the aid of atropin. During the course of these experiments it was soon found that the minimal lethal dose of the serum, which was established practically every time that a batch of experiments was carried out, showed at first a shifting in value with increasing length of sensitization. For example, some guinea pigs, sensitized on November 17, 1909, by the subcutaneous injection of 1 c.c. of horse serum, were tested on December 3 (sixteen days), when 0.6 c.c. heated horse serum injected into the jugular vein killed within five minutes; 0.4 c.c. of the same serum gave only slight respiratory symptoms in another animal. On January 24, 1910 (sixty-eight days), another test was made: 0.2, 0.1, 0.04 c.c. heated horse serum, all killed the guinea pigs within five minutes when injected intravenously; 0.02 c.c. caused powerful respiratory symptoms from which the animal recovered. On June 30, 1910, two hundred and twenty-five days after sensitization, it was found that the sensitiveness of the animal was practically the same as after sixty-eight days: 0.05 c.c. usually killed within five minutes. From these data it will be seen that the sensitiveness of the injected guinea pigs increased until the maximum was reached on or before sixty-eight days, when one fifteenth of that dose killed which was necessary sixteen days after sensitization; and this level was found maintained two hundred and twenty-five days after sensitization. These facts support and supplement respectively some statements by Lewis[21] and by Rosenau and Anderson.[22] Lewis found that the maximum of hypersensitiveness, when tested by the subcutaneous method of introducing the toxic dose, was reached in about three or four weeks. Unfortunately I made no tests between the sixteenth and the sixty-eighth day, and it is very well possible that this maximum occurs well ahead of the latter time. Rosenau and Anderson tested animals after intervals of more than seven hundred and one thousand days and found the animals still extremely sensitive. These authors, however, injected about 6 c.c. of the horse serum intraperitoneally for the toxic dose and their results have therefore only a qualitative value. The results I described briefly above show that there is practically no quantitative fluctuation in the amount of the killing dose of horse serum for at least twenty-three weeks after the maximum sensitization level has been reached.

Action of atropin in immediate anaphylaxis.—This phase of the problem need not be considered here in connection with the present study, for it has been reported in another place.[23] It will be sufficient to state that proof has been advanced that in anaphylaxis the bronchial muscles themselves are sensitized by horse serum and that atropin in proper dosage is able to paralyze the denervated bronchial musculature. I was unable to find any evidence for any rôle which the vagus broncho-motor endings might play in immediate anaphylaxis.

SUMMARY.

A prophylactic injection of atropin sulphate in guinea pigs sensitized by the subcutaneous injection of horse serum saved eighteen out of twenty-five from the lethal effect of the toxic injection; while out of twenty-four adequate controls only six survived. Stated otherwise: The death-rate with atropin was 28 per cent; without atropin it was 75 per cent. These figures show the distinct therapeutic utility of atropin in immediate anaphylaxis.

[21] Lewis: Journal of experimental medicine, 1908, x, p. 6.

[22] Rosenau and Anderson: Hygienic laboratory bulletin, No. 45, 1908, p. 57, and No. 50, 1909, p. 47.

[23] Auer: Proceedings of the Society for Experimental Biology and Medicine, 1910, vii, p. 104; Auer: second communication, Journal of experimental medicine, 1910, xii, No. 5.

Guinea pigs sensitized by the subcutaneous injection of one or two cubic centimetres of horse serum, reach their maximum sensitiveness on or before the ninth week, and this sensitiveness, tested by intravenous injection of the toxic dose, is then practically *quantitatively* maintained for at least twenty-three weeks (the longest interval tested).

THE PREPARATION AND PROPERTIES OF THROMBIN, TOGETHER WITH OBSERVATIONS ON ANTITHROMBIN AND PROTHROMBIN.

By W. H. HOWELL.

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IN a paper from this laboratory by Rettger[1] some facts were given which throw doubt upon the usual view that thrombin is an enzyme or ferment. According to these experiments thrombin in solution is not easily destroyed by boiling, and in its reaction with fibrinogen it does not appear to act as a catalytic or pseudo-catalytic agent. In order to carry these experiments farther it seemed necessary to devise a more satisfactory method of preparing thrombin, and the present paper constitutes a preliminary report upon a new method of isolating this substance. As is well known, the two methods usually employed in physiology to prepare thrombin are, the method of Schmidt, according to which serum is precipitated by excess of alcohol and the precipitate after standing some time under alcohol is dried and extracted with water; and the method of Buchanan as modified by Gamgee[2] and afterward by Lea and Green,[3] which consists in extracting washed fibrin with 8 per cent solution of sodium chloride. Both of these methods, but especially the latter, give solutions which show a strong thrombin action. The solutions obtained by these methods contain much protein in addition to the thrombin. The solution obtained by Schmidt's method contains protein which in the extract as prepared does not coagulate upon boiling. If, however, neutral salts are added to the solution in slight concentration, particularly ammonia salts or salts of the alkaline earths, a heavy precipitate is produced on heating the solution. If the method of extracting the

[1] RETTGER: This journal, 1909, xxiv, p. 406.

[2] GAMGEE: Journal of physiology, 1879, ii, p. 145.

[3] LEA and GREEN: *Ibid.*, 1884, iv. p. 380.

washed fibrin with strong saline is used, the solution contains much protein. When fresh fibrin from the dog is used, and, after thorough washing in water, is digested for twenty-four hours with 8 per cent solution of sodium chloride at 40° C., it is nearly all dissolved, and the filtered solution gives a very abundant precipitate on heating to 57° to 60° C., and usually a second much smaller precipitate when heated further to 76°–80° C. The presence of these large amounts of protein in solution makes it difficult or impossible to study satisfactorily the properties of the thrombin itself. An effort was made accordingly to remove the protein without precipitating the thrombin. A method was finally devised which effected this result, as follows:

METHOD OF PREPARING PURE THROMBIN.

A large quantity of fibrin obtained by whipping pig's blood is thoroughly washed in running water until it is entirely freed from hæmoglobin. The process is tedious, taking several hours, as the fibrin must be continually kneaded in the running water, and the larger masses must be shredded by hand to remove the enclosed hæmoglobin. The white mass finally obtained is minced finely and thoroughly squeezed in cheese cloth, and is then placed in a vessel and covered with an 8 per cent solution of sodium chloride. This preparation is kept in the ice chest for forty-eight to seventy-two hours and is then filtered, first through cheese cloth and then through filter paper. The somewhat viscid solution thus obtained has a powerful thrombin action. To remove the coagulable protein contained in it it was shaken repeatedly with chloroform, adding each time about one half its volume of chloroform, shaking vigorously in the hand or a shaking machine and then filtering through filter paper. At first a heavy precipitate is produced and the liquid above the chloroform is turbid and gives a turbid filtrate. But as the process is repeated again and again, the filtrate becomes clearer and comes through more rapidly. Each time that this filtrate is shaken with new chloroform, the latter quickly forms an emulsion which on standing sinks to the bottom. When the filtrate is entirely clear, or shows only a slight opalescence when seen in bulk, it continues to form an emulsion when shaken with the chloroform, and the process must be continued until after prolonged shaking with fresh chloroform the latter on standing forms a clear layer without evidence of an emulsion. The water-clear filtrate is then examined by heating a specimen to boiling in a test tube. If a turbidity appears, the process of shaking with chloroform must be continued one or more times until a specimen on heating gives no opalescence whatever, even after the addition of some crystals of ammonium sulphate. The clear liquid thus obtained free from all protein coagulable by heat is still rich in thrombin. A few drops added to a solution of pure fibrinogen will cause a firm clot within one or two minutes, the liquid usually becoming first markedly opalescent and then setting to a firm jelly. The process of purifying the thrombin is time-consuming, requiring a number of days, and is attended by a considerable loss of liquid. At each filtration the emulsion of chloroform holds back on the filter some of the solution. The chloroform itself, towards the end at least, when the protein is small in amount, can be preserved and redistilled. If the process is carried out as rapidly as possible, the solution finally obtained contains, as stated above, much thrombin and none of the protein originally present which is coagulable by heat in the presence of neutral salts. This solution, if allowed to stand saturated with chloroform, will, in the course of weeks, lose its coagulating effect on fibrinogen solutions. To keep the thrombin permanently it must be evaporated to dryness as rapidly as possible at a low temperature, 35° to 40° C. My method of doing this has been to place 5 to 10 c.c. of the solution in watch crystals and allow the evaporation to occur during ten or twelve hours in a warm chamber kept at 35° to 40° C. The white residue obtained is mixed with crystals of sodium chloride, and is quickly and completely dissolved on the addition of water. If larger amounts are evaporated and the time of evaporation is thereby much prolonged, the residue may be slightly or deeply colored in yellow and on addition of water a portion of it is found to be insoluble. Corresponding to this change its coagulating effect upon solutions of fibrinogen is diminished or destroyed altogether, so that evidently prolonged exposure to 40° C. in solution brings about a slow decomposition of the thrombin, converting it to a substance insoluble in water. When evaporated in small quantities in watch crystals as described above, the preparations of thrombin are apparently entirely stable. Months afterward the residue dissolved in water shows a prompt and powerful action upon solutions of fibrinogen. So far as my experience goes, such specimens of thrombin may be kept indefinitely. To obtain the thrombin free from the sodium chloride, the solution, before being evaporated, may be dialyzed through collodion tubes. As is well known, crystalloids pass through a collodion membrane very rapidly, so that after a dialysis of five or six hours against a large amount of distilled water, renewed once or twice, the thrombin solution is freed from the sodium chloride, and may then be evaporated to dryness in watch crystals at a low temperature as described. I have been successful in obtaining a number of specimens of thrombin in this way free from sodium chloride. The residue is slight in amount, has a crystalline structure under the microscope, is readily and quickly dissolved in water, and has a powerful coagulating effect upon fibrinogen. Whether or not the crystalline structure shown by these preparations is due to the thrombin or to the presence of minute amounts of inorganic salts was difficult to determine. The crystals had a snowflake appearance, and as far as could be determined they were not entirely destroyed by heating the preparations to the point of carbonization. It seems most probable that the crystals seen in the preparations consisted of minute amounts of salts of some sort not dialyzed off, and that these overlay a granular material also evident which was the thrombin. In preparing my specimens of thrombin I subsequently gave up the process of dialyzing for two reasons. In the first place, it happens in some cases that much of the thrombin disappears during the dialysis, presumably because it is able to diffuse slowly through the collodion membrane. While it is possible to choose such a time for dialysis as will remove the sodium chloride and still leave much thrombin, there is always the possibility that the dialysis may be prolonged so far as to lose most of the thrombin. In the second place, the solutions of thrombin obtained free from sodium chloride by dialysis are somewhat more unstable than the saline solutions when exposed to heat, and are therefore more liable to be decomposed during the process of evaporation. As the presence of the sodium chloride does not interfere with the study of the properties and reactions of the thrombin and seems to confer a greater stability upon its solutions, it was found preferable in the later preparations to evaporate the solutions of thrombin to dryness without previous dialysis.

REACTIONS OF THE THROMBIN.

Solutions of the thrombin before and after dialysis were tested repeatedly by the usual protein reactions and in all cases with positive results. The following reactions were noted:

1. A solution of thrombin remains entirely clear upon boiling even in the presence of salts, including ammonium sulphate (2 to 5 per cent). The thrombin is therefore not coagulated by heat in neutral solutions.
2. It is imperfectly precipitated by a large excess of alcohol.
3. It is precipitated by half saturation with ammonium sulphate, and the precipitate even after standing for several days is readily soluble in water and shows its usual action in coagulating a solution of fibrinogen.
4. It gives a positive biuret reaction.
5. It gives a very distinct tryptophan reaction by the method of Adamkiewicz, using a freshly prepared mixture of sulphuric and acetic acids.
6. It gives a positive xanthoproteic reaction.
7. It gives a positive reaction with Millon's reagent.
8. The reaction for sulphur is negative or very feeble.
9. The reaction with Molisch's reagent is negative.
10. The reaction for phosphorus is negative.
11. With acetic acid and potassium ferrocyanide it gives a faint precipitate; with potassium ferrocyanide alone in neutral reaction no precipitate or turbidity; hence probably it is not a basic protein.
12. Careful addition of dilute ammonia in the presence of ammonia salts gives no precipitate; hence it is not a protein of the histone group.
13. Nitric acid in excess gives a faint turbidity which clears on heating and reappears on cooling. According to these reactions the thrombin must be classed as a simple protein which does not however fall into any of the groups usually described.
14. While the solutions of thrombin prepared as described do not give an opalescence or precipitate when boiled in the presence of neutral salts, it was found that if given an acid reaction with acetic acid boiling causes a turbidity or precipitate unless the solution is very dilute. In the latter case the solution while clear when heated became turbid on cooling, the turbidity disappearing upon heating and returning upon cooling. This reaction naturally gave rise to the sus-

picion that my solutions still contained some trace of the usual blood proteins. Accordingly, I again submitted it to repeated shaking with chloroform, testing each filtrate by boiling a specimen after acidifying with acetic acid. While shaking with the chloroform no longer gave any appearance of an emulsion, it was noticed that after each treatment there was a slight membranous layer upon the surface of the chloroform. By continuing this treatment a filtrate was finally obtained which on boiling after acidifying with acetic acid gave no turbidity upon cooling. Upon testing this filtrate upon solutions of fibrinogen, it was found that the thrombin had entirely disappeared, although the original solution had shown a very strong thrombin action. When tested for protein by means of the biuret, Millon, and tryptophan reactions, the solution gave evidence of the presence of very minute amounts of protein, scarcely detectible. The conclusion to be drawn from this result is that the protein which gave the reaction with acetic acid was not an impurity but the thrombin itself, and that among its distinctive reactions we must include this one, namely, that while incoagulable on heating in neutral solutions it gives in dilute solutions, after acidifying with acetic acid and boiling, a solution which remains clear when hot and becomes turbid on cooling.

15. As stated above, thrombin solutions when saturated with chloroform very gradually lose their activity after a period of weeks or months, depending on the temperature. Aqueous solutions without chloroform or other antiseptic, such as toluol, soon undergo putrefaction, and it is noteworthy, as pointed out by Rettger, that this process at first serves to increase the activity of the thrombin in its coagulating effect on pure fibrinogen. Specimens of washed fibrin suspended in water show a marked thrombin action after putrefaction is well advanced, and this action is maintained for a long period but eventually disappears. It is more striking, perhaps, that specimens of pure thrombin in aqueous solution on undergoing putrefaction, show an increased efficiency as judged by the rapidity with which they coagulate fibrinogen solutions, although eventually, if allowed to stand, the thrombin disappears. This fact suggests that the action of putrefaction on the molecule of thrombin splits off a complex which acts more rapidly on the fibrinogen than the original thrombin. There is some reason to believe that this efficient complex is characterized by the presence of the indol grouping, but the experiments to deter-

mine this point have not yet been brought to a satisfactory termination.

16. **Effect of temperature on the thrombin.** — As stated above, aqueous solutions of thrombin protected from putrefaction slowly lose their efficiency, and this gradual alteration in the thrombin is accelerated by a high temperature. Rettger has shown that aqueous solutions of thrombin prepared by Schmidt's method are weakened but not totally destroyed by short exposures to the temperature of boiling. I have repeated these experiments upon my specimens of pure thrombin and find that those specimens which contain some sodium chloride may be boiled for a few minutes without destroying their activity, although the activity is diminished, as shown by the longer time required to coagulate a given specimen of fibrinogen. On the contrary, solutions of thrombin which have been dialyzed previously to remove the sodium chloride may lose their coagulating effect on fibrinogen completely after boiling for a minute or two. This fact coincides with the result previously mentioned, that dialyzed solutions of thrombin are more liable to alteration than the undialyzed solutions in slow evaporation at a temperature of 35°–40° C. The presence of the sodium chloride makes the thrombin more resistant to the effects of high temperature. Such experiments as the following seem to demonstrate this point: Three specimens of a dialyzed solution of thrombin were taken, 2 c.c. each; one was diluted with an equal amount of water, one with an equal amount of a 2 per cent solution of sodium chloride, and one with an equal amount of an 8 per cent solution of sodium chloride. Each specimen was brought to a boiling temperature for a minute, and then 13 drops of each were added to 4 c.c. of a fibrinogen solution. The specimens were left over night. Those with sodium chloride, 1 and 4 per cent, gave excellent firm clots; the one without sodium chloride gave only an extremely feeble coagulum. The short exposure to boiling had almost but not completely destroyed the thrombin contained in it.

THE QUANTITATIVE RELATIONSHIP OF THROMBIN TO FIBRINOGEN.

The most important factor for consideration in discussing the question of whether or not thrombin acts as an enzyme or catalytic agent is the quantitative relationship between the thrombin and the fibrinogen converted to fibrin. According to the usual belief thrombin acts as an enzyme, and Schmidt has given some experiments intended to show that the thrombin is not used up in the action which it causes, but may be active over and over again after the manner of a catalytic reagent. An examination of Schmidt's experiments will show, as I have pointed out elsewhere,[4] that they are open to the obvious objection that he began his experiments with a large excess of thrombin. To 10 c.c. of a "salt-plasma solution" he added 80 c.c. of a thrombin solution. The seven successive coagulations which he obtained by using the serum of each clot to coagulate a new specimen of salt plasma may have been due therefore to the excessive amount of thrombin with which he started rather than to the fact that the same thrombin acted upon seven successive lots of plasma. His seventh coagulation required four hours, while his first occurred within two minutes. Rettger,[5] on the contrary, describes an experiment in which the amount of fibrin obtained from equal volumes of a solution of fibrinogen was found to vary in proportion to the amount of thrombin added, when the amount of this latter substance was kept below the quantity necessary to coagulate the whole of the fibrinogen. A result of this kind indicates a definite quantitative relation between the amount of thrombin and the amount of fibrin formed such as would not be expected if the thrombin plays the part of an enzyme. In the present investigation the opportunity seemed to be offered to test this point with more care, making use of known amounts of fibrinogen and thrombin. The object of the experiments was twofold, — in the first place to determine whether, when submaximal amounts of thrombin are used, the amount of fibrin formed varies with the amount of thrombin added, and, in the second place, making use of small amounts of thrombin, to see if the amount of fibrin formed varies with the time during which the thrombin acts. The experiments proved to be time-consuming, and only four were completed. One of these was useless owing to the fact that too much thrombin was used. Two of the experiments were successful and gave results which, while positive as regards the questions stated above, brought to light a new and interesting difficulty. The details of these experiments are as follows:

Preparation of the thrombin. — The thrombin used had been prepared by the chloroform method described above. The solution had been evaporated to dryness in watch crystals without previous dialysis. The dry preparations contained, therefore, a quantity of sodium chloride. To estimate the amount of thrombin the following method was used: The contents of each crystal were dissolved in 10 c.c. of water, giving a solution that contained approximately 2 per cent of sodium chloride; 5 c.c. of this solution were evaporated to dryness on the water bath in a weighed platinum crucible, and the crucible with its residue was then heated to 105° C. in an electric oven until the weight was constant. The weight of the total residue, sodium chloride and thrombin, was thus obtained. The crucible was then heated over a small flame until the organic material was incinerated and was again weighed. The difference between the two last weighings gave the weight of the thrombin in 5 c.c. of the solution used. This method did not allow for the weight of any ash that the thrombin itself gives, but the total amount of thrombin used in the experiments, namely, from 0.05 to 0.64 mgm., was so small that this error is negligible. In the first two experiments each cubic centimetre of the solution contained 3.2 mgm. of thrombin, in the third each cubic centimetre contained 2.5 mgm. of thrombin. For the coagulation experiments with fibrinogen 1 c.c. of these solutions was diluted to 10 c.c. with water, and from 2 c.c. to 0.2 c.c. of this diluted solution was used, containing from 0.64 to 0.05 mgm. of thrombin.

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[4] Howell: The coagulation of blood, Cleveland medical journal, January and February, 1910.

[5] Rettger: *Loc. cit.*

Preparation of the fibrinogen. — The fibrinogen used was prepared in all cases from clear plasma obtained from cats which had fasted for twenty-four hours. The method used was a modification of that given by Hammarsten and has been described in previous papers from this laboratory. The plasma obtained by centrifugalizing the oxalated cat's blood was precipitated by the addition of an equal volume of saturated solution of sodium chloride. This mixture was centrifugalized and the supernatant liquid poured off from the precipitate. It is an advantage to centrifugalize the mixture as soon as it is made, otherwise the fibrinogen collects in large flocculi which may rise to the top of the tube during centrifugalization and give difficulty in decanting. The precipitate of fibrinogen in each tube was washed with a half-saturated solution of sodium chloride and then dissolved in a 2 per cent solution of sodium chloride, filtered and again precipitated by addition of an equal volume of saturated solution of sodium chloride. This precipitate was centrifugalized, washed, dissolved in 2 per cent solution of sodium chloride, filtered and again precipitated by half saturation with sodium chloride. After the third precipitation the washed precipitate was finally dissolved in a 0.9 per cent solution of sodium chloride. Sometimes, in fact as a rule, the third precipitate failed to dissolve in the dilute sodium chloride, but in such cases the addition of a drop of a 5 per cent solution of sodium carbonate was sufficient to carry the fibrinogen into solution. This final solution was filtered and was then dialyzed in collodium tubes for about twelve hours against a large volume of a 0.9 per cent solution of sodium chloride, the outside solution being changed once. Solutions of fibrinogen made in this way do not, as a rule, undergo spontaneous coagulation, no matter how long they are kept, although after a certain time they may precipitate. Rettger found it advisable before dialyzing the final solution to treat it with dilute sodium phosphate and barium chloride on the view that the delicate precipitate of barium phosphate in settling out will carry down any remnant of thrombin or prothrombin present in the solution. If care is exercised in the preparations, this additional procedure does not seem to be necessary.

In the experiments made with these preparations a known amount of thrombin was added to given volumes of the fibrinogen solution, and, after standing for definite times, the clot of fibrin formed was twisted out upon a glass rod, placed on a weighed filter paper, washed successively with solutions of sodium chloride 0.9 per cent, cold water, and hot water until the washings were free from chlorides. The fibrin was washed finally with alcohol and ether, dried and then heated in an electric oven at 105° C. to constant weight.

Experiment I. Plasma from 3 cats, fibrinogen prepared and after dialysis two specimens of 35 c.c. each taken for coagulation. To one was added 2 c.c. of the dilute thrombin solution (0.64 mgm. thrombin), to the other 0.5 c.c. (0.16 mgm. thrombin). The mixtures were allowed to stand for twenty-four hours. Solid clots were formed, that with the more thrombin (I) looking much more dense. Clots twisted out with a glass rod, placed on weighed filters and washed and heated as described above.

I. 35 c.c. fibrinogen solution + 0.64 mgm. thrombin
Weight of tube + filter = 11.4118
Weight of tube + filter + fibrin = 11.4543
Weight of fibrin = .0425

II. 35 c.c. fibrinogen + 0.16 mgm. thrombin
Weight of tube + filter = 13.0130
Weight of tube + filter + fibrin = 13.0470
Weight of fibrin = .0340

That is to say, 0.16 mgm. of thrombin gave 34 mgm. of fibrin, while 0.64 mgm. of thrombin gave 42.5 mgm. of fibrin.

Experiment II. Plasma from two cats, somewhat milky from fat. Fibrinogen prepared and after dialysis two specimens of 25 c.c. each taken. To each of these there was added 0.5 c.c. of the dilute thrombin solution (0.16 mgm. thrombin). One specimen was allowed to stand twenty-four hours, the other forty-eight hours.

I. 25 c.c. fibrinogen solution + 0.16 mgm. thrombin, twenty-four hours.
Weight of tube + filter = 12.4878
Weight of tube + filter + fibrin = 12.5045
Weight of fibrin = .0167

II. 25 c.c. fibrinogen solution + 0.16 mgm. thrombin, forty-eight hours.
Weight of tube + filter = 16.0442
Weight of tube + filter + fibrin = 16.0612
Weight of fibrin = .0170

The difference between the two yields of fibrin was only 0.3 mgm. It would seem, however, that in this experiment the fibrinogen solution was much more dilute than in the first experiment, and probably the amount of thrombin added was too large, that is to say, too near the amount necessary to give a maximal formation of fibrin. The experiment is of value, not so much in determining the influence of time upon the amount of fibrin formed with sub-maximal doses of thrombin as in giving an indication of the degree of accuracy in the method used for determining the fibrin.

Experiment III. Plasma from four cats, obtained 200 c.c. of clear plasma, fibrinogen prepared as usual. After dialysis took 3 specimens of 50 c.c. each. Added to one 0.2 c.c. of diluted thrombin (0.05 mgm.) and kept twenty-four hours; to another the same amount of thrombin and kept seventy hours; and to the third 1 c.c. of diluted thrombin solution (0.25 mgm.) and kept twenty-four hours. The last specimen was clotted in two hours, while the first two began to show the initial opalescence at the fourth hour. At the end of twenty-four hours all had reached a final stage, and it was evident to the eye that the clot in the third specimen was much heavier than in the other two.

I. 50 c.c. fibrinogen solution + 0.05 mgm. thrombin, stood twenty-four hours.

Weight of tube + filter = 12.0631
Weight of tube + filter + fibrin = 12.0744
Weight of fibrin = .0113

II. 50. c.c. fibrinogen solution + 0.05 mgm. thrombin, stood seventy hours.

Weight of tube + filter = 15.9820
Weight of tube + filter + fibrin = 15.9922
Weight of fibrin = .0102

III. 50 c.c. fibrinogen solution + 0.25 mgm. of thrombin, stood twenty-four hours.

Weight of tube + filter = 15.5892
Weight of tube + filter + fibrin = 15.6260
Weight of fibrin = .0368

So far as this experiment goes, therefore, it would appear that the larger the amount of thrombin added the greater is the amount of fibrin formed, provided the amount of thrombin is kept below that necessary to convert all of the fibrinogen to fibrin — moreover, with a minimal amount of thrombin the amount of fibrin formed is not increased by longer standing. Both of these facts are opposed to the theory that the thrombin acts after the manner of an enzyme. If the data from the first and the third experiment are compared, they give us the following series:

0.05	mgm.	thrombin	yields	10.75	mgm.	fibrin,	or 1 to 215
0.16	"	"	"	34.00	"	"	or 1 to 212.5
0.25	"	"	"	36.80	"	"	or 1 to 147
0.64	"	"	"	42.50	"	"	or 1 to 66

According to these results the amount of fibrin formed when minimal amounts of thrombin are used is directly proportional to the weight of thrombin, or, in other words, a definite amount of thrombin converts a definite amount of fibrinogen to fibrin. One part of thrombin can combine with or react with 212 to 215 parts of fibrinogen at a maximum. When the thrombin is increased beyond a certain amount, this proportionality disappears. As the thrombin is increased, the amount of fibrin formed is increased but not in direct proportion. Some of the thrombin is apparently inactive, possibly because it is physically adsorbed by the fibrin formed. In order to convert all of the fibrinogen to fibrin it is necessary to add thrombin in much larger amounts than is indicated by the ratio of 1 to 215.

The conclusions to be drawn from the experiments given above are, however, much complicated by the following facts: When the fibrin formed in one of these specimens is twisted out upon a glass rod and the remaining liquid is allowed to stand for a number of hours, a new lot of fibrin forms. If this is removed in time, a third lot of fibrin appears in the course of twenty-four hours. In Experiment III, for example, specimens 1 and 2 gave four successive clots, while specimen 3 gave three successive clots. Thus the original clot in 1 was removed at the end of twenty-four hours. At the end of forty-eight hours a new clot equally voluminous had formed, and when this was removed a third clot of about the same amount had formed at the end of seventy-two hours. Meanwhile specimen 2, exactly similar to 1, which had been standing for seventy hours without being disturbed, had only as much fibrin as in the first clot of specimen 1. Evidently, after a certain amount of fibrin is formed with a given submaximal quantity of thrombin, an equilibrium is reached due to some influence of the fibrin itself, since the removal of this fibrin at once permits the conversion of a new quantity of fibrinogen to fibrin. In specimens 1 and 2, containing each 0.05 mgm. thrombin, four successive and approximately equal clots were formed. After the last clot was removed the liquid gave no further fibrin, but when heated to 60° C. there was an abundant precipitate, showing that the fibrinogen had not all been converted to fibrin. In specimen 3, containing five times as much thrombin (0.25 mgm.), three successive clots were obtained. After the removal of the last one no further clotting occurred, and the liquid when heated to 60° gave only a minute opalescence. Evidently all of the fibrinogen had been converted to fibrin.

A satisfactory explanation or discussion of this phenomenon is not possible without additional experiments to answer the numerous questions that suggest themselves. As in the case of enzymes, the reaction caused by the thrombin is incomplete owing to the fact that the end product of the reaction, the fibrin, inhibits in some way the further action of the thrombin, and this condition of equilibrium is disturbed by the removal of the fibrin. On the other hand, the reaction differs from that of enzymes in two respects. In the first place, in the fact that the portion of the fibrinogen converted to fibrin increases as the amount of thrombin is increased, although not proportionally. If sufficient thrombin is added, all of the fibrinogen is changed to fibrin. In the second place, when a submaximal amount of thrombin is used, the portion of fibrinogen converted to fibrin does not vary with the time that the thrombin is allowed to act. Why the mechanical removal of the fibrin should again start up the thrombin action is difficult to understand. The simplest suggestion, perhaps, is that the fibrinogen combines with a definite quantity of thrombin, or, to be more cautious, a certain amount of thrombin reacts with a certain quantity of fibrinogen, but the fibrin thus formed is able to hold in loose union by mechanical adsorption an additional amount of thrombin. It is this loosely combined thrombin which is liberated by the mechanical process of twisting out and squeezing the fibrin, and is able when set free to precipitate a new lot of the fibrinogen. This suggestion carries with it the conclusion that the maximum ratio of combination by weight of the fibrinogen and thrombin given above, namely, 215 to 1, does not represent a real maximum, since the fibrin held in loose combination a portion of the thrombin which had not reacted with fibrinogen. The maximum ratio of combination of the thrombin would be obtained by determining the total weight of fibrin formed in the successive coagulations. This explanation is not insisted upon, as further experiments may fail to support it. It may be added that the author has observed previously a similar reaction in horse's plasma. If after the plasma is coagulated the clot is broken up and filtered off, the filtrate may clot again, and the process may be repeated a number of times. A somewhat similar process has also been described by the author in connection with the heat coagulation of the proteins of the serum of limulus. When the coagulum obtained at a given temperature is filtered off and the clear filtrate is again heated to the same temperature, a new coagulation occurs, and this process may be repeated several times.

THE ANTITHROMBIN OF PEPTONE PLASMA.

A number of experiments were made with peptone plasma from the dog to determine whether it contains a substance capable of neutralizing the action of thrombin on fibrinogen. The peptone plasma was prepared by injecting into the femoral artery of a fasting dog a 6 to 7 per cent solution of peptone in quantity to yield 0.3 to 0.4 gm. of peptone to each kilogram of animal. Twenty minutes after this injection the animal was bled from the carotid, and the specimen of blood removed was centrifugalized to obtain a clear plasma. In successful cases the plasma remained unclotted for at least forty-eight hours. For the sake of comparison, a specimen of the dog's blood was removed before the injection of the peptone, and was received into an oxalate solution and centrifugalized. The two plasmas from the same animal thus obtained will be spoken of briefly as oxalated plasma and peptone plasma. It was found that the oxalated plasma clotted firmly in a few minutes on the addition of a few drops of thrombin solution; the peptone plasma, on the contrary, as has been observed by others, was not affected by the addition of amounts of thrombin which for the same amount of normal plasma would have meant a large excess. The most decisive experiments were made with fibrinogen solutions to which were added certain amounts of peptone plasma and thrombin. Similar tests were made for the sake of control with mixtures of fibrinogen solutions with thrombin alone or with thrombin and oxalated plasma. These experiments brought out very clearly the positive fact that peptone plasma contains something which prevents thrombin from acting on fibrinogen. The following typical experiment may be quoted:

Fibrinogen solution	2.5 c.c.	This mixture clotted firmly in two minutes.
Sodium chloride solution 0.9 per cent	2 c.c.	
Thrombin solution (2.5 mgm.)	1 c.c.	

Fibrinogen solution	2.5 c.c.	This mixture clotted firmly within two minutes.
Oxalated plasma	2 c.c.	
Thrombin solution (2.5 mgm.)	1 c.c.	

Fibrinogen solution	2.5 c.c.	This mixture showed no sign of clotting after twenty-four hours.
Peptone plasma	2 c.c.	
Thrombin solution (2.5 mgm.)	1 c.c.	

Results of this kind were obtained uniformly and furnish convincing proof that in the peptone blood something is contained which not only prevents the coagulation of that plasma, but is capable of antagonizing thrombin added to it in considerable amounts, or finally prevents thrombin from acting on a solution of fibrinogen. The substance contained in the peptone plasma which prevents its clotting exerts this action no doubt by antagonizing the effect of thrombin. This substance is designated usually as antithrombin, but whether it acts by combining directly with the thrombin has not been demonstrated, although it is made probable by the experiments cited above. That it is an organic substance present in the plasma is indicated by the effect of heating. Peptone plasma heated for ten minutes at 60° and filtered from the heat coagulum of fibrinogen formed at this temperature, still prevents entirely the action of thrombin on fibrinogen, whereas when the plasma is heated to 75°–80° C. and is filtered from the larger heat coagulum it loses completely its power of antagonizing the action of thrombin on fibrinogen. We may say, therefore, that the antisubstance present in peptone plasma is not affected by heating for ten minutes at 60° C., but is destroyed when the temperature rises as high as 75° to 80° C.

An interesting feature of the peptone plasma is the effect of diluting it with water and with physiological saline respectively. In a previous paper the author has called attention to the fact that terrapin's plasma obtained in the fall when the animal is in good condition preparatory to the winter hibernation, does not clot spontaneously. When diluted with water, it clots readily, while diluting with sodium chloride solution 0.9 per cent has no such effect. This fact was used as an argument against the view that the incoagulability of terrapin and bird blood is due to the presence of an antithrombin. My present experience with peptone plasma shows, however, that this argument was not justified. Peptone plasma seems undoubtedly to contain an antisubstance to the thrombin, yet when diluted with water it may be made to clot, while an equal dilution with 0.9 per cent solution of sodium chloride has no such effect. The amount of dilution with water necessary to cause clotting in the peptone plasma varied in my experience in the different specimens of blood. The difference depends, no doubt, on the amount of antisubstance present in the plasma. With specimens of peptone plasma containing an amount of antisubstance sufficient to prevent the plasma from clotting for at least forty-eight hours, dilution with five times its volume of water caused clotting after a few hours, while an equal dilution with saline solution remained unclotted for over twenty-four hours at least. If this reaction in peptone plasma is associated with the presence of an antithrombin, then we may infer that the normal terrapin's (and bird's) plasma which shows a similar reaction may likewise owe its non-coagulability to the presence of an antisubstance. It may be added that dilution of oxalated plasma with water does not cause coagulation.

Experiments upon prothrombin. — It seemed probable that the chloroform method of isolating thrombin might be used successfully to isolate its antecedent substance, the so-called prothrombin. For this purpose the blood of a fasting cat was received into oxalate solution, and, after centrifugalizing, the clear plasma was dialyzed against 0.9 per cent salt solution for twenty-four hours to get rid of the excess of oxalate. The dialyzed plasma was then shaken with chloroform for one to two hours and filtered. The filtrate clotted. This result showed that the method is not applicable to the isolation of prothrombin, but it is interesting as proving that prothrombin can be converted to thrombin in a calcium-free solution. Whatever may be the mode of action of calcium salts in activating prothrombin to thrombin, it is evident that other conditions may effect the same activation, although in a less effective manner.

A further experiment was made upon prothrombin to determine in how far it is precipitated from oxalated plasma in the preparation of fibrinogen. Mellanby has assumed an intimate relationship between the fibrinogen and prothrombin in normal plasma which it seemed possible to test by a simple experiment. For this purpose clear oxalated plasma was prepared from cat's blood. From this plasma the fibrinogen was precipitated by the addition of sodium chloride until the liquid was three quarters saturated. The precipitated fibrinogen was centrifugalized off, was washed twice with half-saturated solution of sodium chloride, and was then dissolved in a 2 per cent solution of sodium chloride. It was found that this solution did not clot spontaneously after twenty-four hours, that it did not clot upon the addition of an equal volume of 0.9 per cent solution of sodium chloride, but that it did clot quite promptly upon the addition of a few drops of a solution of calcium chloride. Evidently, therefore, in the precipitation of the fibrinogen some prothrombin was carried down with it, and it was interesting to inquire whether it was all thus precipitated with the fibrinogen. To determine this point the original plasma, after the removal of the fibrinogen, was dialyzed against 0.9 per cent solution of sodium chloride to reduce the amount of sodium chloride in it, and was then tested for the presence of prothrombin. It was found that this dialyzed liquid added to a solution of pure fibrinogen gave no clot, but that if a little calcium chloride was also added coagulation occurred in a short time. It is scarcely necessary to add that calcium chloride added to a pure solution of fibrinogen has no effect. From these two experiments it would seem that the prothrombin, as we should expect, is carried down in part only by the precipitated fibrinogen. In the usual method of purifying the fibrinogen by several (3) successive precipitations the prothrombin may be gotten rid of completely, but it is obvious that all preparations of fibrinogen used in experiments upon coagulation should be examined in regard to this point by testing their coagulability after the addition of calcium chloride.

Experiments upon the production of thrombin from other proteins. — It has long been stated that thrombin (fibrin ferment) may be produced from other proteins especially during the process of putrefaction. Some of these statements rest undoubtedly upon a confusion between thrombin action proper and zymoplastic or thromboplastic action. Many substances will facilitate the reaction between fibrinogen and thrombin or fibrinogen and prothrombin, but no substance except thrombin or some derivative compound is capable of converting a pure solution of fibrinogen to fibrin. On such solutions zymoplastic substances are entirely without action. But the conclusion arrived at in this investigation, namely, that the thrombin is a protein body of probably simpler structure than the usual animal proteins, made it probable that it might be produced from such proteins by processes of hydrolysis capable of being controlled. The experiments on this line have not been wholly successful and will be described very briefly. Attempts were made to prepare the thrombin from fibrin by acid hydrolysis, pancreatic hydrolysis, and by putrefaction. The product in each case was treated to isolate basic proteins; that is to say, it was precipitated while hot with a hot acid solution of phosphotungstic acid, the precipitate was filtered off, washed with 5 per cent sulphuric acid containing some phosphotungstic acid, and treated with hot solution of barium hydrate. After filtration the excess of barium was removed by careful additions of dilute sulphuric acid and the neutral filtrate was evaporated to dryness at a low temperature and its action was tested upon pure solutions of fibrinogen. These solutions caused always a precipitation of the fibrinogen but not a coagulation, except in the case of the putrefied fibrin. This preparation treated as outlined above gave solutions which in some cases when added to a fibrinogen solution threw down at once a gelatinous precipitate resembling a clot. It would seem that the thrombin present in quantity in the putrefied solution of fibrin had been carried through the process given above without wholly losing its power of throwing down fibrinogen in the form of a gelatinous precipitate. The process differed from that of normal coagulation in the immediate production of a precipitate. Insufficient quantities allowed to act for a long time gave no effect, while further addition threw down a gelatinous precipitate at once. A somewhat more promising result was obtained from the action of bacteria. Through the kindness of Dr. W. W. Ford cultures were made in bouillon and Dunham's solution with the bacillus coli communis, proteus vulgaris, and bacillus of Metchnikoff. These cultures were passed through a Berkefeld filter and were then tested upon a solution of pure fibrinogen with their normal alkaline reaction and after neutralization with dilute acetic acid. None of the specimens had clotted after five hours, and it was then necessary to leave them unobserved for thirty hours. At the end of that time the culture of the colon bacillus in broth, both neutral and alkaline, had caused a clot in the fibrinogen, while the culture of the same bacillus in Dunham's solution and the cultures of the other bacteria were without action. It has not been possible as yet to follow this suggestion further.

Intravenous injection of thrombin. — It would seem probable that the very powerful specimens of pure thrombin prepared by the chloroform method might cause a distinct effect if brought directly into the circulation of a living animal. A single experiment of this kind gave, however, entirely negative results. A small dog was used weighing 3750 gm. It was anæsthetized with morphia and ether, and, while connected with a kymographion for the registration of blood pressure, solutions of thrombin were injected into the femoral vein.

Three injections were made, — 16 mgm. thrombin at 10.04 A. M.; 16 mgm. at 10.10 A. M., and 32 mgm. at 10.20 A. M. None of these injections produced any effect upon the pulse rate or blood pressure. Dr. Duke kindly determined the coagulation time of the blood during the experiment from drops of blood drawn from the ear. The normal coagulation time was from five to seven minutes. After the second injection there was a slight but temporary lengthening of this period to ten minutes. The blood plates examined in Wright's solution showed no indications of agglutinating. The results of the injection were therefore entirely negative, and indicate that the body can normally neutralize and render harmless quite large doses of thrombin introduced suddenly into the circulation.

SUMMARY OF RESULTS.

1. A new method is described for preparing thrombin free from admixed protein. The method consists in treating washed fibrin with dilute salt solution (8 per cent sodium chloride) to dissolve the thrombin and then precipitating the coagulable protein by repeated treatments with chloroform.

2. Pure thrombin gives reactions which indicate that it is a simple protein. It does not contain phosphorus or sulphur, gives positive reactions with the biuret, millon, and especially the tryptophan tests, is not coagulated by boiling in neutral solutions, is very readily soluble in pure water, is completely precipitated unchanged by half saturation with ammonium sulphate. In weakly acid solutions (acetic acid) it gives on heating a solution which shows a turbidity on subsequent cooling.

3. Thrombin when allowed to stand in solution for long periods, protected from putrefaction by the addition of chloroform, gradually undergoes an alteration and eventually loses its power of coagulating fibrinogen. This change is hastened by a high temperature. Solutions of thrombin allowed to undergo putrefaction show first an increased power of coagulating fibrinogen followed after a long period by a complete loss of coagulating effect.

4. Solutions of thrombin containing some sodium chloride may be heated to the boiling temperature without losing completely their coagulating action on fibrinogen. Dialyzed solutions of thrombin

are destroyed more completely and rapidly by exposure to high temperatures.

5. Thrombin when dried at low temperatures and protected from moisture in a desiccator may be kept indefinitely.

6. Thrombin probably does not act upon fibrinogen after the manner of an enzyme. Increasing amounts of thrombin give increasing amounts of fibrin, although in decreasing proportion. The weight of fibrin produced by a given submaximal amount of thrombin is not affected by the time during which the thrombin is allowed to act. When minimal amounts of thrombin are used, one part of thrombin can convert at least 215 times its weight of fibrinogen to fibrin.

7. In the non-coagulable peptone plasma of the dog there is contained an antisubstance (antithrombin) which prevents the action of thrombin on fibrinogen. This antisubstance is not destroyed by a temperature of 60° C., but is destroyed at 75° to 80° C. Dilution with water causes spontaneous coagulation in peptone plasma; dilution with saline solution (0.9 per cent sodium chloride) has no such effect.

8. Prothrombin may be converted to thrombin in solutions free from calcium salts.

9. Large amounts of pure thrombin may be injected into the circulation of the living animal without any noticeable effect.

THE LATENCY OF KNEE-JERK RESPONSE IN MAN AS MEASURED BY THE THREAD GALVANOMETER.

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RECENT developments in the use of the thread galvanometer in registering physiological reactions, and especially the results obtained by Piper [1] in the use of this instrument for redetermining the velocity of nerve conduction in man, have made it seem worth while to reinvestigate the nature of the phenomenon of knee jerk.

The work of Waller [2] and of Gotch [3] seemed at the time to yield conclusive evidence that the response of the extensor cruris to the tapping of the patellar tendon was a direct response of the muscle to mechanical stretching, and not to reflex nervous stimulation. This evidence consisted mainly of a comparison of the measured delay in the response to tapping (1) with that obtained by direct mechanical percussion or electrical stimulation of the muscle, (2) with that obtained by reflex stimulation or by estimation of the time required for such reflex action. Waller found a latency of .03 to .04 second in the response to tendon tapping in man. The same muscle upon direct galvanic stimulation showed a latency of .02 second. In the rabbit these values were much smaller and more nearly equal, being .0080 and .0076 second respectively. Gotch, working also on the rabbit, reduced these figures to .005 second, the latency to tendon tapping being no longer than the latency to direct electrical stimulation. Franz [4] reports the latency to tendon tapping in the normal human to be as much as .04 or .05 second.

Waller concluded that the difference of .01 or .02 second between his latencies in man was not enough time for a reflex action. Indeed the total time was less than that Exner[5] found for eyelid reflex, namely, .06 second.

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[1] PIPER: Archiv für die gesammte Physiologie, 1908, cxxiv, p. 591.

[2] WALLER, A. D.: Brain, 1880, iii, p. 179; Journal of physiology, 1891, xi, p. 384.

[3] GOTCH: Journal of physiology, 1896, xx, p. 322.

[4] FRANZ: The American journal of insanity, 1909, lxv, p. 471.

Both Waller and Franz used tambours and levers to record their knee-jerk responses. The delays they measured may be due partly to delays in their recording apparatus, a source of error common also to Exner's experiments on eyelid reflex.

During the work in progress in this laboratory with the thread galvanometer Professor Howell has expressed a desire to know what the latency of the knee-jerk reaction would be if measured by this instrument. He furthermore wished to know what the character of the action current of the muscles involved would be if the latter could be led off to the thread galvanometer. Would the latency be enough to allow for a reflex action? Would the action current be a simple vibration, or a series of vibrations of the thread?

While a number of points need still to be determined by experiments before the question as to reflex can be answered, there are at this writing two points in the problem which can be answered definitely: (1) *The latency of the knee-jerk response in man, as shown by the thread galvanometer, is about .011 second;* (2) *The action current obtained by leading off from non-polarizables placed over the thigh muscles never produces anything more than a single diphasic deflection of the galvanometer thread.*

The latter statement must be modified by saying that other vibrations of the thread are found late in the record. But they are purely "tetanus" rhythms produced by the subject who reflexly tries to control the swinging of his leg after the first extension. This occurs whether the foot be weighted or not. The extra waves are always late in the record and clearly have nothing to do with the knee-jerk phenomenon proper.

METHOD.

The small electro-magnet thread galvanometer made by Edelmann of Munich was used in these experiments. The movements of the thread were magnified by projection of the thread's shadow, which was photographed by means of the revolving photographic apparatus also made by Edelmann.

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[5] EXNER: Archiv für die gesammte Physiologie, 1874, viii, p. 526.

The method of leading off to the galvanometer used was that described by Piper [6] in his work on voluntary tetanus in man and employed later by him in measuring the time of nerve conduction in man.[1]

The point of stimulation on the photographic record was produced by allowing the handle of the hammer to cast its shadow on one margin of the sensitive film just as the hammer struck the patellar tendon. As the hammer rebounds from the tendon, its shadow is removed from the film. The point of stimulation on the photograph thus takes the shape of an inverted V. A tuning fork of 100 D. V. was used to mark time, the shadow of one arm being likewise photographed.

The delay of the thread galvanometer in responding to electrical changes is, as Einthoven [7] has shown, practically nil. Nevertheless, the one used in this laboratory has been tested for this point. To do this, a lever connected with the make and break key of the galvanometer current was so placed as to allow its shadow to be photographed along with that of the galvanometer thread. Examination of the records obtained showed no measurable difference between the signal points and the beginning deflection of the thread. The galvanometer itself has no latency.

It is thus seen that by using such a method errors due to mechanical contrivances are greatly reduced. Indeed the delay thus measured must represent the purely physiological delay of the knee-jerk response.

RESULTS.

The following table shows the measurements of the latencies thus obtained from the subject C. H.:

No. of photograph.	Date. May (1910)	Latency in seconds.	No. of photograph.	Date May (1910)	Latency in seconds.
1	7	.013	7	11	.009
2	7	.014	8	11	,010
3	9	.012	9	12	.009
4	9	.011	10	12	.008
5	10	.013	11	14	.015
6	10	.012	12	15	.010

[6] PIPER: Archiv für die gesammte Physiologie, 1907, cxix, p. 301; Zeitschrift für Biologie, 1908, l, p. 393.

[7] EINTHOVEN: Annalen der Physik, 4, 1904, xiv, p. 182; 1906, xxi, p. 483.

The average of these results is .01133 second.

Two observations were made upon the writer as subject. The latencies were .015 and .013 second respectively.

Examples of the photographic records are reproduced in Fig. 1. They should be read from right to left. The time mark is a tuning-fork of 100 double vibrations per second. On one of the records an additional time mark of a Jaquetz in fifths is to be seen. The upper trace in each record is that of the galvanometer thread; the lowest trace, with the heavy inverted V, marks the moment of tapping the tendon.

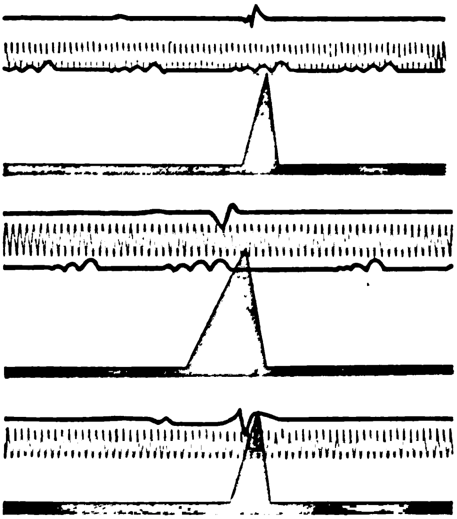

FIGURE 1.

DISCUSSION.

What are the physiological latencies that make up the total delay in knee-jerk response? If in man, as in rabbit, the sudden pull given to the stretched tendon by tapping stimulates the muscle fibres directly to contraction, then our delay would consist of (1) the time required for the blow to produce an adequate stimulus by the sudden additional stretching it causes, (2) the latency of the muscle contraction itself after receiving adequate stimulus. The motor nerve endings in this case may or may not be involved.

If, on the other hand, a reflex arc be involved in the total delay, then the several latencies in the delay are (1) the time required for an adequate stimulus to develop in the sensory endings in the tendonous tissue; (2) the time of mere nerve conduction to spinal centre and back to extensor cruris; (3) the time consumed in the cord, the "synapse"; (4) the latency of the motor endings together with the time required to develop an action current in the muscle tissue itself.

Let us consider the former case first, — the knee jerk is not a reflex action. Of the two possible latencies listed for this case the first is unknown. For the second we may refer to the measurements of Gad and Heymans,[8] who found it to be .005 second in curarized frog's muscle at 35° temperature; and to the work of Burdon-Sanderson,[9] who found a latency of .003 second in curarized frog's sartorius. The latter result was obtained for the action current of the muscle as shown by the capillary electrometer. The delays recorded by Gotch[3] and Waller[2] upon direct stimulation probably include the delay in the motor nerve endings as well as latency of contraction. The figures are .005 second and .0076 second respectively, and were obtained from the extensor cruris in rabbits. The result obtained by Piper[1] on man is perhaps of the most value for present purposes. On stimulating the flexores digitorum of man through the median nerve at the elbow by the unipolar method, this author found a latency of .00442 second. Allowing for the conduction time over the nerve path, this author calculated the combined latency of motor ending and action current in these muscles to be nearly .0036 second. It should be remembered that it was the action current and not the actual contraction of thigh muscles that was recorded in the present experiments. This fact makes the use of Piper's figure here especially valuable, but, on the other hand, it must be remembered that Piper's action current was excited by electrical means, while that obtained in the knee-jerk experiments was excited by mechanical means. It may be recalled, however, that Gotch found no difference in the latencies obtained by these two methods.

Let us now take up the second assumption, namely, that knee jerk in man is a true reflex. The total delay in this case, as stated above, may consist of four elements. The first, that of the sensory endings, we know nothing about and must for the present therefore neglect.

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[8] GAD und HEYMANS: Archiv für Anatomie und Physiologie, Physiol. Abt. Suppl. 1890, p. 59.

[9] BURDON-SANDERSON: Journal of physiology, 1895, xviii, p. 117.

The second latency, that of nerve conduction, we may take from Piper's measurement for the median nerve in man. This author found the velocity of conduction to be about 120 metres per second for this nerve. His work has been repeated in this laboratory and corroborated. From Nicolai's[10] experiments on the olfactory nerve of the pike, we may assume for the present that the velocity of conduction of both motor and sensory nerves is the same. Let us assume, then, that the velocity of conduction of both motor and sensory paths in the thigh of man is 120 metres per second.

In the subject, C. H., of these experiments, the distance from the tapping point on the patellar tendon to the lumbar spine was 70 cm.; from the lumbar spine to the middle of the thigh, 50 cm. This makes a total of 120 cm. for nervous conduction. At the rate of 120 metres per second the time required for this distance would be just .01 second. If we assume that the combined latency of motor endings and development of action current in the extensor cruris is the same as Piper found for flexores digitorum, we have then the additional latency of .0036 second.

Buchanan[11] estimated the delay in the cord of the frog for spinal reflex to be .012 second at 15° and .022 second at about 5°. These figures indicate a temperature coefficient for the synapse in the frog to be nearly 1.85 for differences of 10°. This fact, in connection with what we already know of the temperature coefficients of physiological processes generally,[12] and especially of those processes involving nerve centre,[13] gives us a basis for calculating the latency of synapse at the temperature of the human body. Let us assume that figure for the same process in man. Using the formula, $Q_{10} = \left(\frac{k_0}{k_1}\right)^{\frac{10}{t_1 - t_0}}$, then, the latency of the synapse would be about .003 second at 37°.

Summing up, the total delay required in a spinal reflex in man to correspond to the knee-jerk phenomenon obtained on the subject C. H. would be as follows:

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[10] NICOLAI: Archiv für Anatomie und Physiologie, Physiol. Abt. Suppl. 1905, p. 341.

[11] BUCHANAN: Quarterly journal of experimental physiology, 1908, i, p. 1.

[12] SNYDER: This journal, 1908, xxii, p. 309.

[13] VERNON: Journal of physiology, 1895, xix, p. 18; WAKELIN: Journal of physiology, 1903, xxix, p. 369; BAGLIONI: Zeitschrift für allgemeine Physiologie, 1907, vii, p. 249; BABAK und ROCEK: Archiv für die gesammte Physiologie, 1909, cxxx, p. 477.

1. Time for total nervous conduction	.010 second
2. Time for synapse	.003
3. Time for latency in motor ending and muscle	.0036
Total delay for a true reflex[14]	.0166
Average delay for knee jerk as found by thread galvanometer	.0113

Thus it is seen that the number found by experiment is smaller than that which we ought to expect for a reflex action by nearly 32 per cent. On the other hand, if Piper's latency in the arm is any index of what the latency in thigh muscle would be upon mechanical stimulation, this number is too large by .008 second (or nearly 200 per cent) to admit of the knee jerk being interpreted as a direct response to the tap.

The fact that no indication of muscle rhythm was to be found in the galvanometer records argues somewhat in favor of the latter view. For the fact that independent muscle rhythm has been observed does not alter the value of such rhythms when observed during spinal (reflex) stimulation.

Is it possible that in man, unlike the rabbit, there is a considerable difference between the latency of muscle contraction when provoked mechanically and electrically?

To answer this question in part the writer carried out the following experiment upon himself, the subject, C. H., no longer being available.

Using a "medical" battery of silver-chloride dry cells the m. rectus femoris was stimulated by the unipolar method. The stimulating electrode was applied to the point indicated by both Siemssen[15] and Boruttau[16] to produce an extension of the knee resembling that occurring in knee jerk. The point was found easily by trial, and carefully demarcated. Non-polarizables were placed over the muscle above the point of stimulation and in the galvanometer circuit, whereby not only the action current, but also, as shown by Piper,[1] the stimulating signal could be recorded. Indeed the whole method is that Piper used on the m. flexores digitorum in man.

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[14] It is of interest to compare this figure with that estimated by François Franck for spinal reflex, namely, .017 second. Quoted after Sherrington: Integration of the nervous system, 1906, p. 87.

[15] Siemssen, Hugo v.: Electricität in der Medizin, Berlin, 1887, p. 276.

[16] Boruttau und Mann: Handbuch der gesammte medizinische Anwendung der Electricität, Leipzig, 1909, i, p. 455.

The latency for the m. rectus femoris as thus measured on the writer varied between .0025 and .0036 second. The latter figure is that given by Piper for muscles of the fore-arm, and was used in the above estimate for the latency of muscle and motor endings.

This latency of m. rectus femoris when compared with the delay of knee-jerk response (.0113 second) is remarkable. For it shows that if knee jerk be in fact response to direct mechanical stimulation, then the difference of the latencies of the muscles involved, when subjected to direct excitation by these two methods, is enormous.

In the light of the work of Waller and of Gotch, as quoted above, this is most unlikely to be the case. However, this explanation cannot be entirely abandoned until we have the results of experiments on the thigh muscle of man which has been stimulated for an absolute certainty directly and mechanically and through its tendon.

The objection may be raised that in the above estimate data were taken not only from different individuals, but from different kinds of animals.

It is to be regretted that for synapse we have nothing nearer man than frog — and theory. As to the measurements on man we have them all in the records for the subject C. H.; that is, we have his conduction time over median nerve and his muscle latency for flexores digitorum. And these are indeed somewhat less than those given by Piper.

Still other modifications of our estimate may be made. As Professor Howell has suggested, if knee jerk be a true reflex it may be that the sensory stimulus originates in the muscle spindles rather than in end organs of the tendon. Allowing for this, the length of the conduction path of the median n. in C. H. becomes 105 cm. instead of 120 cm.

Again the estimate would be appreciably altered if 2.5 were used as temperature coefficient instead of 1.85 in calculating the delay of the synapse. In view of the fact that this coefficient is more nearly that of authors who furnish exhaustive data on temperature influence upon nerve centre, and in view of the paucity of Miss Buchanan's observations upon this point, surely no violence can be done in making the substitution.

With these changes the estimate for delay of true reflex of the extensor cruris in the subject C. H. becomes:

	Second
(1) Time for nerve conduction for 105 cm. at rate of 127 metres per second	.00945
(2) Time for action current latency of muscle and motor nerve endings	.00211
(3) Time for synapse, calculated from Buchanan's data and Q_{10} = 2.5	.00192
Total delay in response for true reflex in the subject C. H.	.01348
Longest delay in knee-jerk response observed on C. H.	.015
Shortest delay in knee-jerk response in C. H.	.008
Longest latency of m. rectus femoris observed on writer	.0036

A comparison of these figures would seem to leave little doubt but that knee jerk in man is a true reflex. However compelling these data may be, the author wishes to postpone final decision, awaiting more experimental evidence concerning the nature of the excitation produced by tapping the patellar tendon, as well as the actual delay at the synapse in man.

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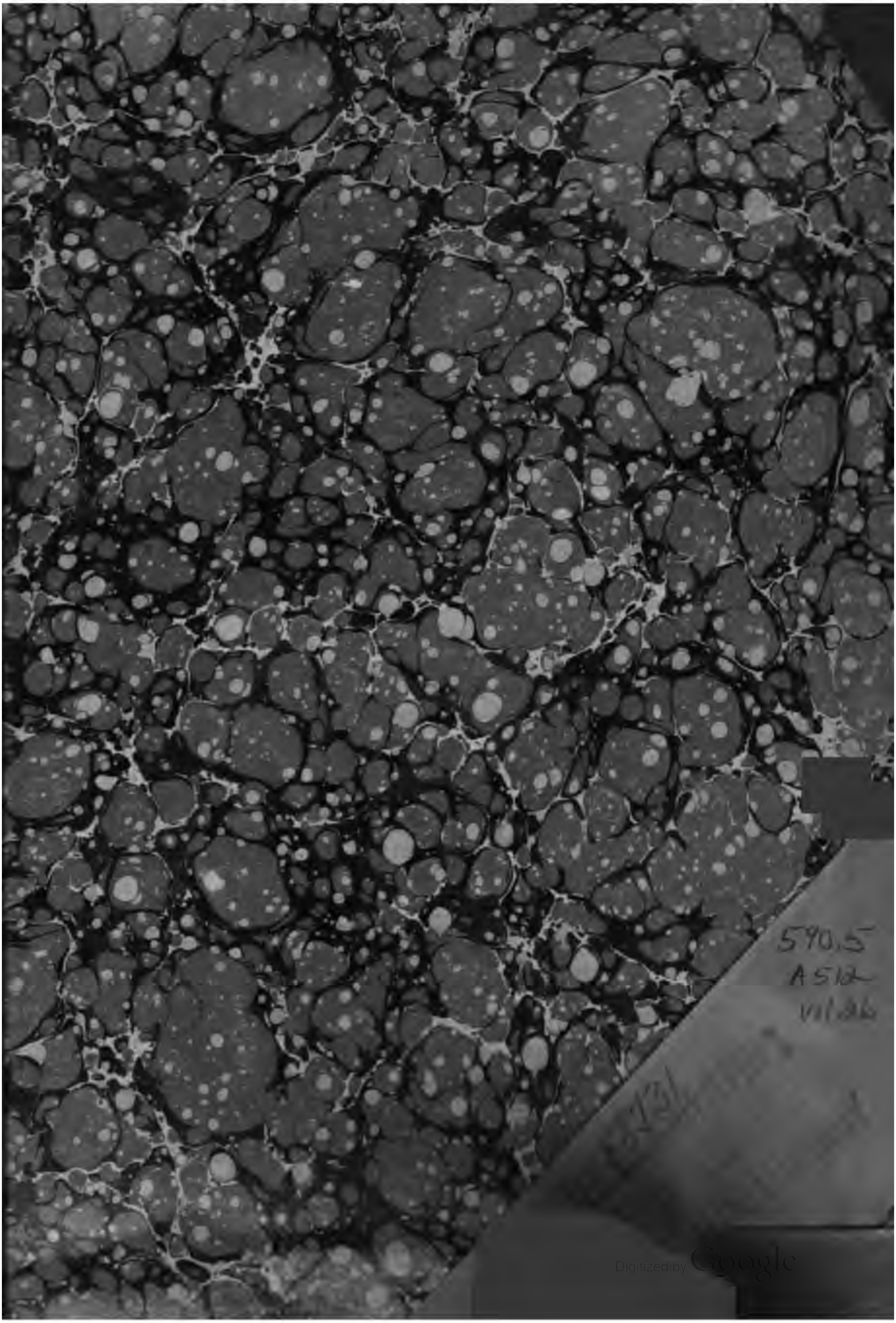

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